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OBSERVATIONS ON THE REPRODUCTIVE STRUCTURES OF *ANACARDIUM OCCIDENTALE*

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The important economic species *Anacardium occidentale* Linnaeus (1753), the source of cashew nuts, is native in the American tropics. It was introduced in the orient apparently in the sixteenth century. It is common in southern India both in cultivation and as an escape. This region is the principal commercial source of cashew nuts (the United States is the main market).

Material was collected in the State of Andhra, in southern India, and sent by Professor J. Venkateswarlu, of Andhra University, and by Professor T. S. Sadasivan, Director, University Botany Laboratory, Madras, to both of whom I am deeply obliged. Certain regretted gaps in the observations arise from difficulties of collection inherent in the material.

Observations

Anacardium occidentale is a small tree having alternate leaves, simple, entire, obovate, rather large; terminal clusters of abundant flowers, yellow or striped with pink; gray nuts, of approximately the shape of inverted commas, of lengths exceeding 3·5 cm, solitary on fleshy pedicels which may be swollen to dimensions of several centimeters. The pericarp — the nut shell — is chambered, and the chambers contain a poisonous oil. Upon harvest, the shells are cracked by roasting, and the embryos, which are the cashew nuts, are extracted. The fleshy pedicels are intensely astringent, yet have some local use as food.

INFLORESCENCE — The flowers are borne in terminal panicle-like clusters. Each branch of the cluster bears a terminal flower subtended immediately by two or more bracts. From the axils of the bracts grow further bracted flower-stalks. Thus the ultimate cluster of flowers is a typical monochasical cyme, and the apparent panicle is actually a thyrse.

FLOWERS — The flowers are polygamous, being of two types, perfect and staminate. As a general rule, the terminal flower of each cyme is perfect and the lateral ones are staminate. The two types are of essentially the same structure; the perfect flower will first be described, and the distinctive features of the staminate flower will then be noted.

Each perfect flower (Fig. 1) stands upon an obconic pedicel a few millimeters long. The summit of the pedicel, the receptacle, bears five separate oblong-acute imbricate sepals, erect and overlapping so as to form a tube about as long as the pedicel. Five linear-acute petals, alternate with the sepals, more than 10 mm long, spring from within the tube of sepals. At anthesis they are recurved, bringing the tips to the level of the receptacle. The outer surfaces of the sepals and petals are pubescent with simple hairs which are unilaterally swollen at the base, with the effect that they are inclined toward the tips of the organs which bear them (Fig. 2).

The stamens, normally ten in number, are united at the base of the filaments to form a tube about 2 mm long. The outer surface of the tube, and the bases of the filaments above it, are pubescent with minute glandular hairs (Fig. 3). The stamens are not arranged in a circle, but in an ellipse, as indicated by the cross-section of the tube. Nine stamens are short, their anthers included in the cylindrical part of the floral envelope. The tenth, located at one end of the ellipse, has a filament, much stouter and longer

than the others, upon which the anther, larger than the others, is exserted. The anthers are basifixed, of quite normal structure, bilobed, dehiscent through a slit between the two pollen sacs of each lobe.

No disk is present. The pistil is of dorsiventral symmetry. The ovary is laterally compressed, with one and larger than the other; the larger end is directed toward the major stamen. The ovary contains a single locule and a single apotropous ovule, the funiculus ascending from the floor of the locule in the smaller end of the ovary[1]. The single style springs from the distal margin of the ovary. It is long and slender, tapering to a very slightly expanded stigma.

In the staminate flower (Fig. 6) the obconic pedicel and the cylindrical part of the perianth are more slender than in the perfect flower. The major stamen protrudes to a greater distance. The pistil is more or less considerably reduced, often to a more sliver 2-3 mm long. The well-known figure of *Anacardium pumilum* by Engler (1896), showing a staminate flower in which the major stamen grows in the place of the pistil, does not represent the structure of *A. occidentale.*

Vascular System of the Flower — A cylinder of a small number of bundles enters the base of the pedicel. These bundles divide freely, in radial planes, to produce an expanding cone of many bundles. As a normal character of Anacardiaceae, most of these have large resin ducts in the phloem. In the receptacle, three from among these bundles run out to each sepal, and one, which forks promptly into three, to each petal.

At levels barely above those at which the perianth bundles become recognizable, the ten stamen bundles — one to each stamen, each of them in the mid-plane of one of the perianth parts — also become recognizalble. These stamen bundles are of varied origin. They may be branches of the perianth bundles or other bundles of the outer whorl which bend laterally into the planes of the perianth parts; or uniting pairs of such bundles; or they may come up through the outer part of the pith. The bundles to the sepalad stamens become distinct at a lower level than the ones to the petalad stamens.

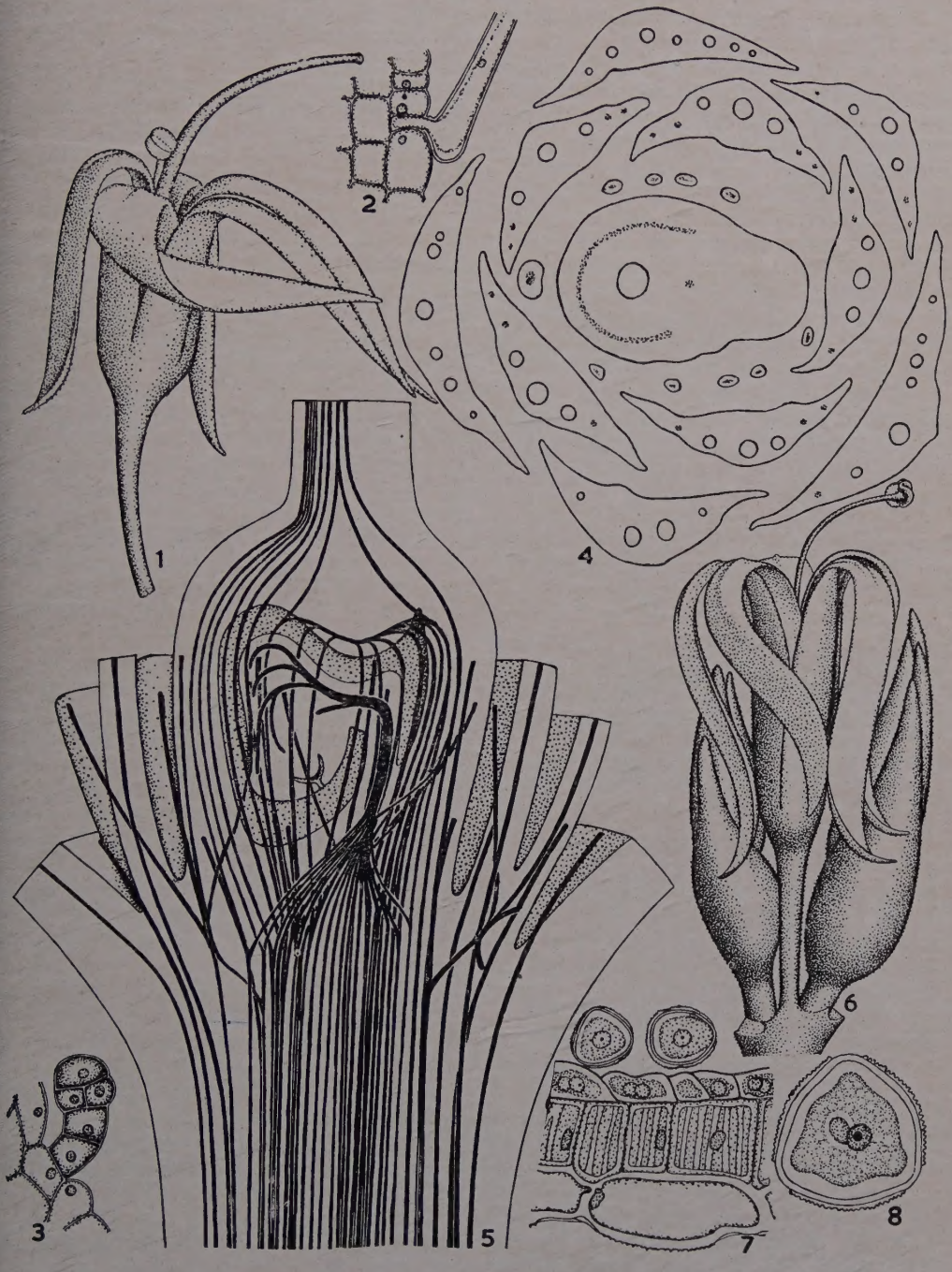
In the pedicels of perfect flowers (Fig. 5), large numbers of small bundles, generally without resin ducts, are scattered in the pith. These originate as branches from the bundles of the outer cycle, and run vertically up the pedicel while the outer bundles diverge. In the receptacle, most of the outer medullary bundles divide into inner and outer branches. The outer branches, together with bundles or branches from bundles of the outer cycle in the pedicel, supply the ovary wall. The number of ovary wall bundles is large, of the order of sixty. In the large end of the ovary the bundles are closely packed, almost a continuous layer; in the small end they are more widely scattered (in Fig. 5, representing one-half of a flower divided in the sagittal plane, I have drawn about a dozen ovary wall bundles instead of about thirty, in order to avoid hiding other features). At the summit of the ovary, many of the ovary wall bundles unite to form two transverse strands, one on each side of the ovary. The remainder, together with branches from the transverse strands, run up the style, where they are crowded into a trough-shaped band, horse-shoe-shaped in cross-section, open toward the small end of the ovary.

In the centre of the style there is a strand consisting of several columns of darkly staining cells (Fig. 4). This is the path for the pollen tubes.

The inner branches of the outer medullary bundles, together with the unbranched inner medullary bundles, run together to form one stout bundle, not in the middle of the receptacle but on the side toward the small end of the ovary. The bundle thus formed supplies the ovule.

In the pedicels of staminate flowers there are no medullary bundles. There is no ovule supply, and other bundles to the pistil are feebly developed or absent.

1. There is a gross mistake in my paper on *Pistacia* (1955): the median style is said to stand above the large end of the ovary, the fact being that it stands above the small end. The misstatement is explicable if not excusable by the fact that when it was written I had already begun to study *Anacardium*, in which the style actually belongs to the large end of the ovary.



FIGS. 1-8 — Fig. 1. Complete flower. × 5. Fig. 2. Hair from sepal. × 400. Fig. 3. Glandular hair from base of filament. × 400. Fig. 4. T.s. complete flower. × 25. Fig. 5. Diagram of the course of bundles in one half of a complete flower divided in the sagittal plane. × 25. Fig. 6. Staminate flower and two buds. × 5. Fig. 7. T.s. wall of anther. × 400. Fig. 8. Mature pollen grain. × 900.

ANTHERS — Small and large anthers are of the same microscopic structure. The cells of the hypodermal layer (endothecium) are radially ribbed. The tapetum is of the secretory type, the cells becoming binucleate and subsequently being absorbed (Fig. 7). The pollen grains are three-grooved, with the exine between the grooves finely pitted, and are binucleate at maturity (Fig. 8).

OVULE AND EMBRYO SAC — The funiculus of the single ovule of each perfect flower arises laterally and extends upward along the ovary wall at the small end. The massive main body of the ovule, attached laterally to the summit of the funiculus, extends to the opposite end of the locule, having the raphe above, and the micropyle below, opening towards the base of the funiculus.

The upper margin of the ovule is curved and concave upward. A bulge of tissue of the ovary wall projects down into the curve, separating two little upward extensions of the locule, the one in the large end of the ovary being decidely the larger (Fig. 9). The strand of specialized tissue which guides the pollen tubes down the style ends in the middle of the bulge, above the centre of the raphe.

The integument is a single massive structure. A certain grooving at the free end, and variations in staining, indicate that it is actually two integuments completely fused. Several branches of the bundle in the raphe curve down within it. There is a massive nucellus, roughly distinguishable from the integument by heavier staining, but without a definite boundary except at the free micropylar end (Fig. 10). The embryo sac is deeply imbedded.

Of the development of the ovule and embryo sac, very little has been seen. This fact results from difficulties of collection. A branch of the tree which is large enough to bring to maturity a single fruit will bear perhaps a score of cymelets, each of several flowers, the oldest perfect and the remainder staminate. If only one among these flowers is to yield a fruit, the development of the others must be checked at some point. The interruption of development appears most often to occur by abortion of the megaspore mother cell. My experience has been this: in sagittal sections of more than one hundred ovules, only two or three have shown scattered stages of normal development. In all others, while the integument and nucellus have been normal, the megaspore mother cell has become a shrivelled scrap. An associated difficulty of collection is this: as flowering begins, all flowers which are to produce normal pistils are nearly fully developed; all young buds are of staminate flowers.

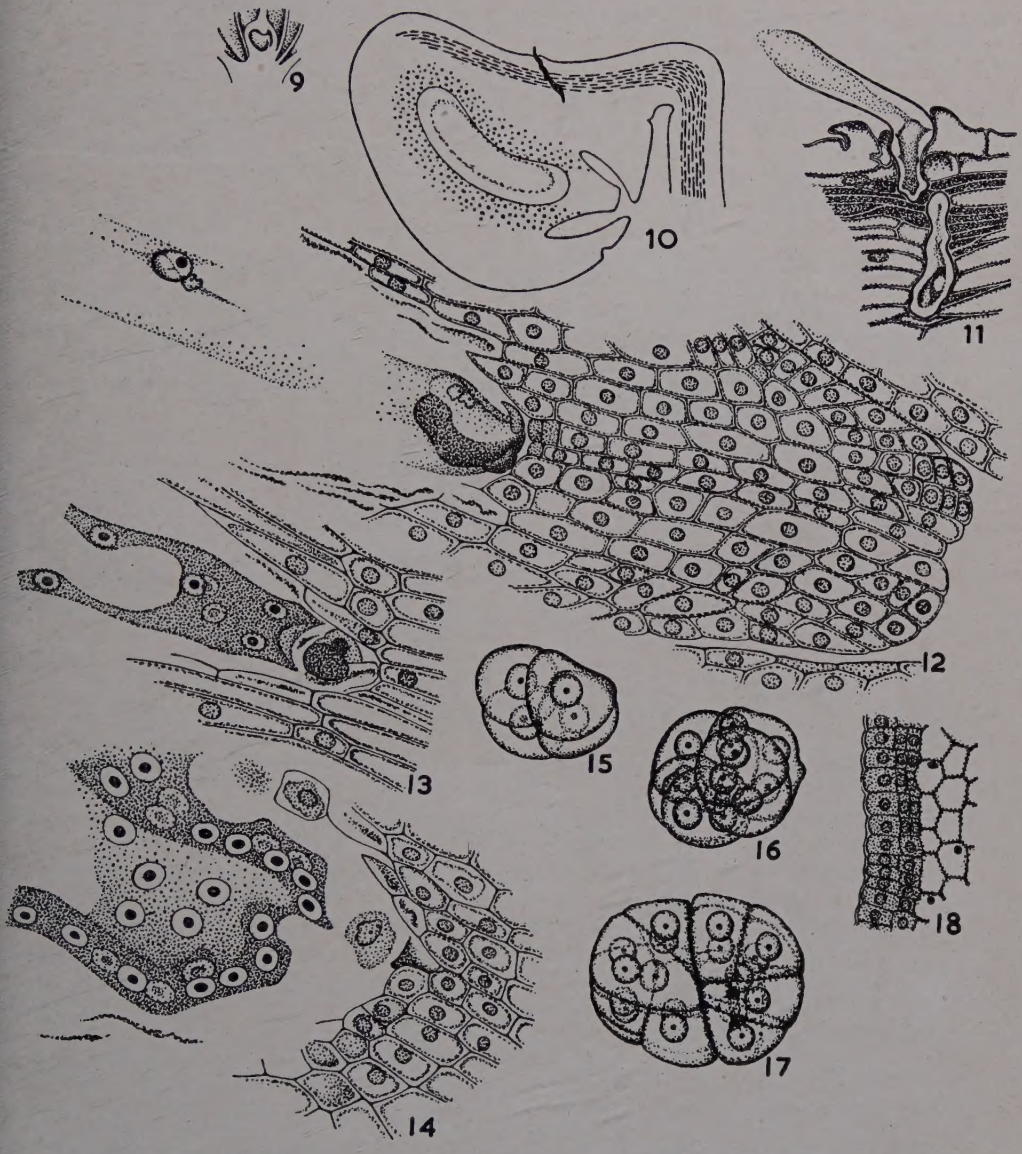
The manner of development of the nucellus can be recognized even when the ovule is mature. The distal part of the nucellus consists of radiating rows of cells undoubtedly produced by periclinal division in a hypodermal archesporium. After the nucellus has reached nearly full size, a few periclinal divisions occur also in the epidermis (Fig. 12).

FERTILIZATION — The pollen tubes, having followed to its end the previously described strand of specialized tissue in the style, grow across a small gap to the middle of the raphe and penetrate it (Figs. 10, 11). Their further path has not been observed. Nevertheless, it is certain that fertilization is apogamous.

Double fertilization, the union of one sperm nucleus with the egg nucleus and of the other with the large nucleus formed by union of the polar nuclei, was seen in a single preparation (Fig. 12).

DEVELOPMENT OF FRUIT AND SEED — At anthesis, the ovary and the ovule are placed approximately transversely to the long axis of the flower (Fig. 9). After fertilization, the ovary grows in all parts, but most considerably in the larger end, which contains the aforementioned relatively large upward extension of the locule. This end curves upward into the position formerly occupied by the (now fallen) style, with the effect that the curved mature fruit lies approximately in the axis of the flower (Fig. 19). As the pericarp grows in thickness, the ovary wall bundles grow radially to the form of flanges.

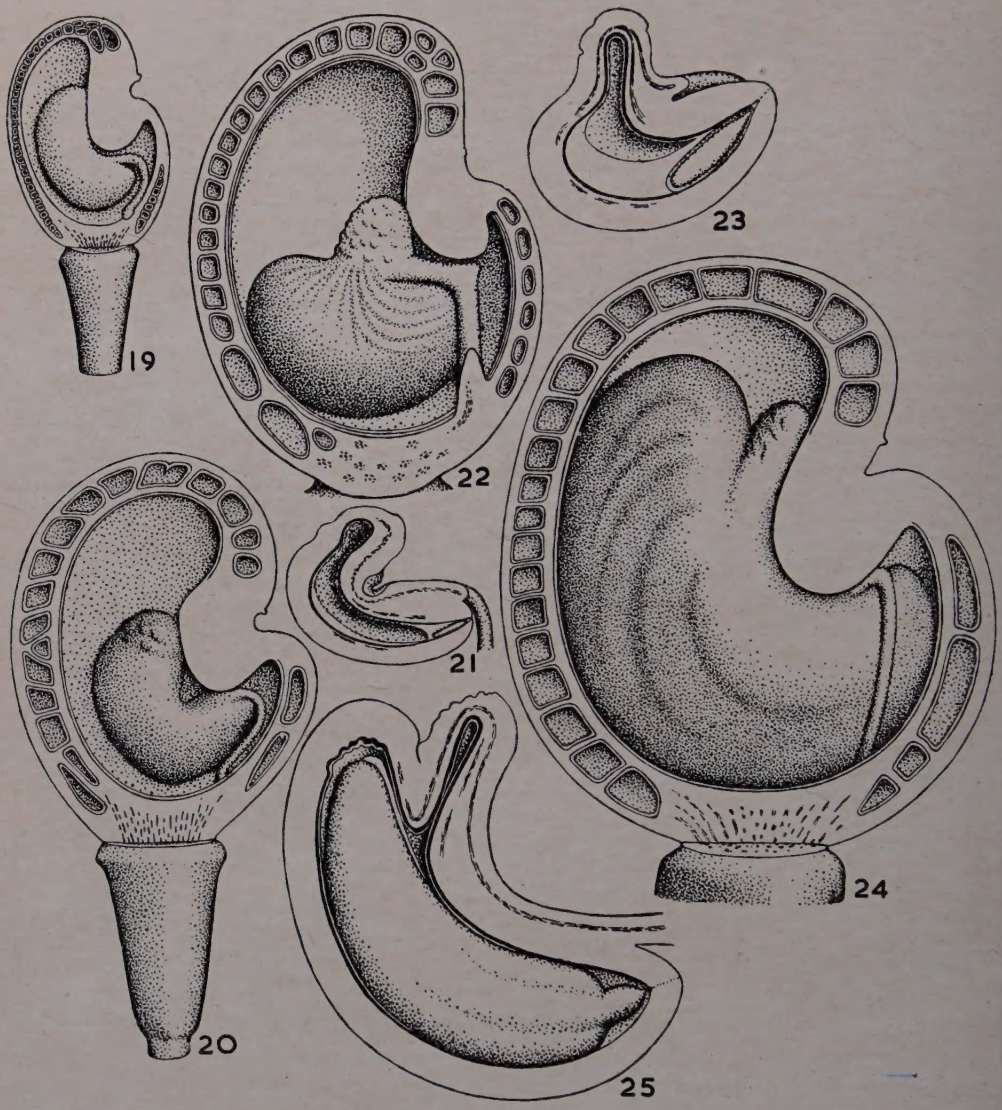
The ovule enlarges at first less rapidly than the ovary, with the effect that it does not fill the locule. The early growth of the ovule consists largely of the



FIGS. 9-18 — Fig. 9. L.s. base of flower. × 2·5. Fig. 10. L.s. ovule showing penetration of pollen tube. × 25. Fig. 11. Two pollen tubes penetrating the raphe. × 400. Fig. 12. L.s. ovule showing double fertilization. × 400. Fig. 13. Zygote and nuclear endosperm. × 400. Fig. 14. Same, slightly older. Figs. 15-17. Young embryos, the micropylar direction being to the right. × 900. Fig. 18. T.s. inner surface of ovary at the stage of zygote and nuclear endosperm. × 400.

extension and curving upward of the chalazal end (Figs. 19, 20, 21).

The endosperm is of nuclear type. The nuclei distribute themselves in one layer along the periphery of a large central vacuole except in the cytoplasmic mass at the micropylar end (Figs. 13, 14). The heavily staining cells of the inner part of the nucellus appear to fall apart as the seed coat enlarges, and the endosperm grows by digesting them. The tip of the nucellus, a conical body of radiating



FIGS. 19-25 — L.s. developing fruits and seeds. × 2·5. Figs. 19, 20, 22, 24. Successive stages of the developing fruit. Figs. 21, 23, 25. Sections of the developing seed respectively from Figs. 20, 22, 24.

rows of cells, is not digested, but survives as a plug in the micropyle. This plug is conspicuous until some time after the beginning of the development of the embryo. Eventually, the integument, becoming massive and growing far beyond the plug, completely closes the micropyle and renders the plug inconspicuous.

The zygote remains undivided until the ovary reaches a length of about 10 mm, and the ovule a length of about 5 mm. Remnants of the synergids and pollen tube, seen at first as scraps of heavily staining material, gradually disappear.

A few very early embryos have been seen. From the four-celled stage (Fig

15), it appears that the first division is by a nearly transverse, slightly oblique, wall, and that each of the two cells divides by a longitudinal wall at right angles to the sagittal plane of the fruit and seed. The sequence of further divisions was not ascertained. It appears that these divisions cease to occur concurrently at a very early stage: globular embryos which appeared to contain respectively 14 and 18 nuclei were seen (Figs. 16, 17). There is no suspensor: all cells derived from the zygote become parts of the embryo proper. The micropylar end becomes a brief hypocotyl. Two cotyledons, their ventral surfaces meeting in the sagittal plane of the fruit and seed, develop at the other end. The cotyledons grow forth along the lower side of the endosperm, the side, that is, which is away from the raphe.

At about the time of the division of the zygote, the free nuclear endosperm at the micropylar end enters the cellular phase. Then a mass of cellular endosperm forms along the outer curve of the seed more or less half way to the chalaza. The liquid in the middle of the endosperm persists above it. The film of nucleate protoplasm of the endosperm which bounds this body at the chalazal end and on the side toward the raphe does not become cellular. It remains unchanged for some time and then becomes moribund.

When cellular endosperm has formed along the outer curve of the seed, this area of the seed begins to bulge. Subsequently, the bulge grows forth and up past the chalaza, forming a large pocket into which the cotyledons grow (Figs. 20-25). The chalaza grows no more. It is pushed aside to become a knob in the middle of the concavity of the seed; eventually it is more or less completely crushed. The endosperm remains perceptible for a long time in the axis of the knob. As the integument develops into a seed coat, many more bundles become differentiated. Most of these originate from the end of the original main bundle of the ovule, in the chalaza. They continue to be recognizable, when the chalaza has become converted into a lateral knob, running down all sides of the knob and radiating from its base.

As fruit and seed approach maturity, the seed grows more rapidly than the pericarp; its end grows up into the end of the locule and fills it.

At the latest stage seen in preserved material (Fig. 25), the embryo forms a smooth curve from the tips of the cotyledons to the tip of the hypocotyl. In mature cashew nuts, however, the hypocotyl is recurved against the cleft between the cotyledons on the upper side. The bending of the hypocotyl into an up-and-back position occurs, therefore, at a late stage of development. Perhaps the mechanical pressure of the proximal ends of the cotyledons against the inside of the seed coat contributes to produce the bending.

While the seed is developing, the pericarp is of course growing to its full size, largely by elongation in the area which was originally the large end of the ovary, and which is now the upper end of the fruit. The mass of tissue which originally projected into the upper edge of the locule is now lateral, greatly enlarged, pressed against the inner curve of the outline of the seed.

The characteristic chambers of the pericarp make their first appearance, before fertilization, in the upward-facing part of the large end of the ovary. There is in this area much vascular tissue in an early stage of differentiation. The chambers originate in this tissue, schizogenously, that is, by a drawing apart of cells. They grow, however, lysigenously, by progressive collapse of the cells surrounding them. Each chamber is lined, at every stage after the earliest, by a layer of material which is yellow in color and which resists staining. This material consists of moribund cells. Apparently these cells accumulate some oily substance, and are then digested, with the exception of the oily substance, which becomes part of one large globule in each chamber.

Where the chambers first appear, in the area which lies eventually above the middle of the inner curve of the outline of the fruit, they occur in more layers than one. Throughout the remainder of the pericarp there is a single layer of chambers arranged in regular vertical columns. Mention was made above of

a stage not long after fertilization, in which the ovary wall bundles are radially enlarged to the form of flanges. A large column of tracheids becomes differentiated in the inner margin of each flange; one column of chambers forms in the middle; further tracheids become differentiated in strands scattered between or outside of the chambers. The number of columns of chambers in the circumference of the pericarp is about sixty, agreeing with the number of ovary wall bundles.

At about the time of fertilization, the cells of the inner epidermis of the pericarp undergo periclinal divisions, producing in some areas two to three layers of cells (Fig. 18). The cells of the innermost layer become radially elongated, to a length of about 0.2 mm, and form in the ripening fruit a conspicuous palisade lining the locule. The cells of the adjacent layer, undergoing anticlinal but not periclinal divisions, produce a minuscule second palisade about 0.02 mm thick. The cells of the third layer, where this is present, become indistinguishable from the ground tissue.

Discussion

The contributions, on floral structure and embryology of Anacardiaceae which are known to me, include the following: Grimm (1912); Juliano (1932); Juliano & Cuevas (1932); Maheshwari (1934); Srinivasachar (1940); Copeland *et al.* (1940); Copeland (1955, 1959); Sharma (1954); Sachar & Chopra (1957) and Kelkar (1958a, b). I am particularly acquainted, of course, with *Toxicodendron*, *Schinus*, and *Pistacia*, upon which I have worked. All three belong to the tribe Rhoideae.

The following paragraphs present comparisons, in various features, between *Anacardium* and other Anacardiaceae.

The inflorescence of *Anacardium*, as of other Anacardiaceae, is basically cymose.

The flowers of *Anarcardium* are polygamo-monoecious, as are also those of *Mangifera* (Sharma, 1954). *Anacardium* and *Mangifera* belong to the tribe Mangifereae. The flowers of most Rhoideae are dioecious.

The flower of *Anacardium*, like that of most Rhoideae, has ten stamens, but differs in having the stamen nearest the large end of the ovary differentiated by exceptionally great size. *Mangifera* has normally five stamens. Here also the stamen at one end of the ovary is the largest; it is, in fact, usually the only fertile stamen.

The stamens of *Anacardium*, as of other Anacardiaceae, are of normal structure, having a ribbed endothecium and a tapetum of secretory type, the cells becoming binucleate (Srinivaschar, 1940, found the tapetal cells of *Spondias* to become 4-nucleate). The mature pollen grains are 3-grooved (*Pistacia* differs), with fine pitting between the grooves, and become binucleate.

Anacardium has no disk, and the bases of the filaments are united. The disk of *Mangifera* is evidently not the same thing as the disk of *Schinus* and *Rhus*: it lies outside of the bases of the stamens, and can be construed as consisting of the bases of the filaments, united and greatly swollen.

The pistils of most Anacardiaceae (not those of tribe Spondieae) are dorsiventral. In the Rhoideae, there is a median style above the small end of the ovary and two lateral styles above the large end, in which the ovule is attached. In *Anacardium* the ovary bears only the median style, and this is located above the large end of the ovary, the ovule being attached in the small end.

The ovaries in the genera observed (excepting *Spondias*) are unilocular bearing a single ovule. The bundle supplying this ovule originates (except in *Schinus*) by fusion of bundles from all sides of the receptacle.

In *Anacardium* as in the Rhoideae, the several or many ovary wall bundles undergo anastomoses at the summit. The bundles to the style or styles are for the most part not simple continuations of the ovary wall bundles. At least some of them are branches from transverse bundles connected to wall bundles in both ends of the ovary.

The figures of Sharma can be interpreted, it is believed, as showing essentially the same structure in *Mangifera*. In this genus, the style stands above the end of the ovary in which the ovule is attached

(Sharma, 1954; Juliano & Cuevas, 1932). Its median bundles, however, come from the other end, and the trough of vascular tissue which ascends it is open toward the end toward which it is attached. Its structure, compared with that of *Anacardium*, is as though it had been moved, with unchanged orientation, from one end of the ovule to the other.

The ovules of *Spondias* (Juliano, 1932) and of *Schinus* and *Toxicodendron* have two distinct integuments. In *Pistacia* and *Anacardium* the integument is a single body exhibiting features which indicate that it is a fusion product of two integuments combined. *Anacardium* does not exhibit the specializations of the outer integument, or of the outer part of the single integument, which have been noted among the Rhiodeae. *Mangifera* appears to agree with *Anacardium* in the points just mentioned.

In the nucelli of *Schinus* (Copeland, 1959), *Toxicodendron* (Copeland & Doyel, 1940), *Lannea* (Kelkar, 1958b), *Rhus* (Kelkar, 1958a), *Pistacia* (Copeland, 1955) and *Anacardium*, it is known that a layer of hypodermal cells at the distal end undergo periclinal divisions to produce rows of cells radiating from a single deeply seated embryo sac. In *Anacardium*, the cells of the epidermis also undergo periclinal divisions; this is not known to be true of the other genera mentioned.

The pollen tubes of *Spondias* and *Semecarpus* are believed to enter the ovule through the micropyle (Srinivasachar, 1940). Those of *Toxicodendron*, *Pistacia*, and *Anacardium* are known to enter through the chalaza or raphe. The occurrence of double fertilization may be considered established in *Spondias* and *Anacardium*.

In every genus carefully studied, the pericarp grows rapidly before the seed begins to do so, and the seed grows rapidly before the embryo begins to do so (Kennard, 1956).

The endosperm of Anacardiaceae is of the nuclear type. In *Anacardium* it becomes organized as cells first about the embryo and then along the side away from the raphe part of the distance from the micropylar to the chalazal end. The remainder of the endosperm — a body of lifeless liquid and a film of nucleate protoplasm which bounds this where cells do not form — does not ever become cellular, but is crowded out as the embryo grows. This manner of growth of the endosperm, an evident specialization, has not been observed in other Anacardiaceae.

The first division of the zygote in Anacardiaceae appears usually to be by a slightly oblique wall. A few cells at the micropylar end produce a brief suspensor, not definitely set apart, in *Schinus* and *Rhus* (Kelkar, 1958a). There is no suspensor in *Spondias* (Srinivasachar, 1940), *Anacardium* (Srinivasachar, anticipating present observations), *Mangifera* (Juliano & Cuevas, 1932), *Pistacia*, or *Semecarpus* (Srinivasachar, 1940).

The hypocotyl is turned at a right angle toward the cleft between the cotyledones on the side toward the raphe in *Anacardium* and in the Rhoideae. As to the stage of development at which the bending occurs, I know only that it is at a relatively early stage in *Pistacia*, and at a very late stage in *Anacardium*.

The growing seed of *Schinus*, *Pistacia*, and *Anacardium* has been seen to become bulged on the side away from the raphe; the bulge grows forth beyond the chalaza, producing a large pocket into which the extremities of the cotyledons grow.

The internal epidermis of the ovary of *Anacardium* undergoes periclinal divisions, producing in fruit two distinct layers of columnar cells bounding the locule. A similar course of events produces in *Toxicodendron* two definite layers and an indistinct third one, and in *Schinus* and *Pistacia* three definite layers.

In making these comparisons, one is attempting to apply microscopic observation upon the reproductive structures to the classification of the genera of a family of more than seventy genera (Engler, 1896; Barkeley, 1957). It will require much more labor to show, either that the accepted system, that of Engler, is essentially sound, or else that it is radically to be revised.

The present observations show *Anacardium* and *Mangifera* definitely allied and distinguished from the Rhoideae by the single style and the growth of one stamen much larger than the others. The pattern

of the vascular bundles in the pistils of these genera is not at all characteristic of a simple pistil. It is remarkably similar to that of the tricarpellate pistils of the Rhoideae. The appearance is, that the pistil of *Anacardium* and *Mangifera* is a tricarpellate pistil so reduced as to give the appearance of a simple pistil.

Summary

The small tree of *Anacardium occidentale* bears flowers in cymes which are grouped into thyrses. As a rule the first flower of each cyme is perfect, and the remainder staminate.

The flower has normally five sepals, five petals, and ten stamens. There is no disk. The filaments are united for a short distance at the base. They are arranged in an ellipse. One stamen, standing at one end of the ellipse, is much larger than the others. All anthers are fertile. They have a ribbed endothecium and a tapetum of secretory type, the cells becoming binucleate. The pollen grains are tricolpate, finely pitted on the surface between the grooves and binucleate.

The pistil (fully developed only in the perfect flowers) is dorsiventral. The large end of the ovary is toward the large stamen. The vascular supply to the single style comes mostly from this end.

There is a single locule. The vascular supply to the single ovule is formed by bundles running together from all sides of the receptacle. The funiculus ascends from the floor of the locule in the small end of the ovary and bears the apotropous ovule in a transverse position with the chalaza in the other end. There is one integument, evidently formed by the complete fusion of two. The nucellus grows by periclinal division in a hypodermal layer of cells. Certain periclinal divisions take place also in the epidermis of the nucellus.

The pollen tube enters the ovule through the raphe.

The fruit, with its locule, grows largely by elongation at the large end, which curves up into the area previously occupied by the style. At maturity, the pericarp contains a layer of approximately cubical chambers, which originate schizogenously but grow lysigenously, whose contents are said to be poisonous. The locule is lined by two layers of columnar cells, the inner elongated to macroscopic dimensions, the outer microscopic.

The ovule, in becoming a seed, enlarges at first largely by elongation of the raphe, while curving upward at the chalazal end, to follow the curvature of the fruit and locule. The endosperm is nuclear. It becomes cellular only about the embryo and along the side away from the raphe. Presently, the growing seed bulges out in this area, and the bulge grows up past the chalaza, crowding it aside. The cellular endosperm, with the enclosed embryo, grows into the area originally occupied by the non-cellular part, and largely eliminates the latter.

The embryo has no suspensor. At a late stage of development, the hypocotyl becomes turned sharply upward.

Most of these characters of *Anacardium* are known in other Anacardiaceae. The vascular system of the pistil is notably similar to those of the tricarpellate pistils of tribe Rhoideae. It is suggested, and the suggestion is extended to *Mangifera*, that the pistils of these genera are tricarpellate, but so reduced as to have the outward appearance of simple pistils.

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MORPHOLOGICAL AND EMBRYOLOGICAL STUDIES IN THE FAMILY LORANTHACEAE — VII. *ATKINSONIA LIGUSTRINA* [CUNNINGH.] F. V. MUELL.

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The monotypic genus *Atkinsonia ligustrina* (Cunningh.) F. V. Muell. is a small evergreen tree endemic to Australia. *Atkinsonia* was generally believed to be a non-parasitic plant but in 1952 McKee proved it to be definitely parasitic on the roots of neighbouring plants such as *Acacia intertexa* Sieb., *Platysace linearifolia* (Cav.) Norman, *Leptospermum attenuatum* Sm., *Monotoca scoparia* R. Br., *Caustis* sp. and *Dillwynia ericifolia* Sm. (Menzies & McKee, 1959).

A brief report on the embryology of *Atkinsonia* has already been published (Garg, 1958).

Material and Methods

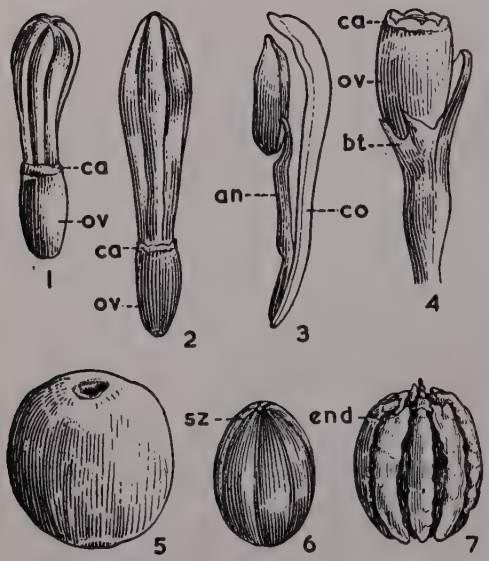
The present account is based on the study of material kindly sent by Professor H. S. McKee of the University of Sydney, for which I am very grateful to him.

The tertiary-butyl-ethyl alcohol series was used for dehydration of floral parts. The hard fruits had to be softened in hydrofluoric acid (diluted in 70 per cent alcohol) for 8-15 days. Sections were cut 9-20 microns thick and stained in safranin-fast green. The female gametophyte, endosperm and embryo were also studied from whole mounts.

Observations

FLORAL MORPHOLOGY — The hexa- or septamerous flower (Fig. 1) is subtended by three bracts (Fig. 4). The corolla is choripetalous and the stamens epipetalous. The latter are adnate at the base only so that the major part of the filament remains free (Fig. 3). The anthers are dorsifixed and versatile (Fig. 3). The calyculus is represented by a 6- or 7-lobed rim (Figs. 1, 2, 4). In contrast to the pseudoberry which is characteristic of the family, the fruit is a pseudodrupe (Figs. 5, 6).

VASCULAR ANATOMY OF FLOWER — Depending upon whether the flower is



FIGS. 1-7 — (*an*, androecium; *bt*, bract; *ca*, calyculus; *co*, corolla lobe; *end*, endosperm; *ov*, ovary; *sz*, stony zone). Figs. 1, 2. Young and mature buds. × 10. Fig. 3. Epipetalous stamen with dorsifixed versatile anther. × 10. Fig. 4. Ovary with three bracts; one of them is usually larger. ×10. Fig. 5. Mature fruit. ×5. Fig. 6. Same; after removal of outer leathery coat and viscid layer to expose the stony zone. × 5. Fig. 7. Deeply-lobed endosperm after removal of stony zone. × 6.

hexa- or septamerous, the basal region of the ovary receives six or seven vascular traces from the stele of the pedicel. The following account is based on the floral anatomy of a septamerous flower. The seven bundles divide and become arranged in two rings (Figs. 9-12). The outer ring consists of seven groups of three bundles each and the inner of five to seven bundles (Fig. 12). The bundles of the inner ring fuse in such a manner that only four carpellary traces are recognizable (Fig. 13). On this basis the gynoecium can be considered as tetracarpellary.

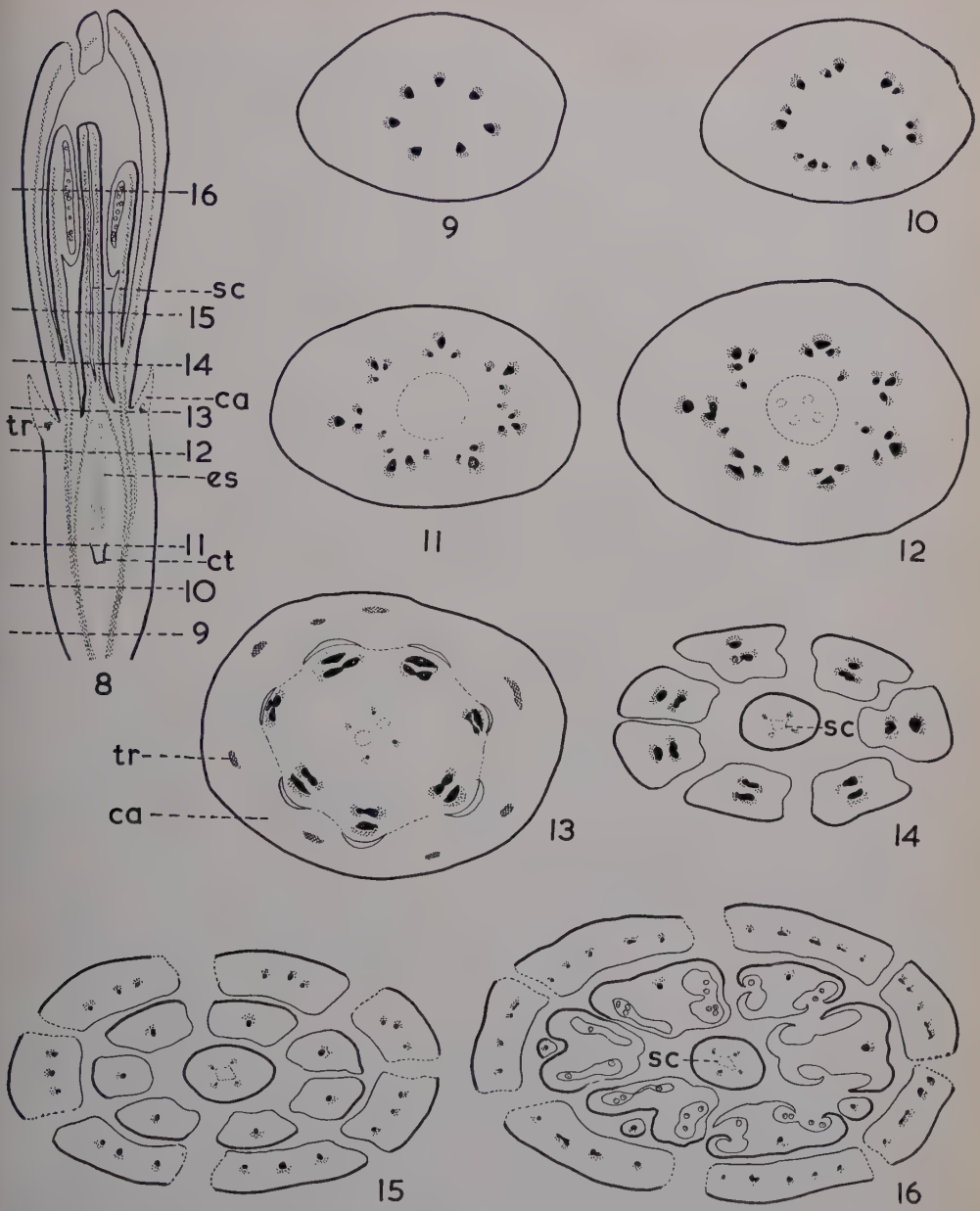
Of the three bundles in each group of the outer ring, the median one represents the petal trace while the two lateral bundles are the staminal traces. Approximately at the level of the calyculus the two staminal traces of each group fuse into a single strand which lies inner to the petal trace (Fig. 13). The staminal trace continues unbranched into the filament whereas the trace entering the petal branches into several bundles (Figs. 14-16). The calyculus does not receive any vascular trace from the main stele but a group of isolated tracheids differentiates in each lobe (Figs. 8, 13, 17-20).

MICROSPORANGIUM AND MALE GAMETOPHYTE — The dithecous anther dehisces by two longitudinal slits (Fig. 21). The wall of the mature anther consists of the epidermis, fibrous endothecium, middle layer and the tapetum. The epidermis persists at maturity but becomes very much flattened (Fig. 22).

Due to paucity of the material earlier stages of the development of the anther could not be studied. The pollen grains are spherical (Figs. 22, 23) and this is an important deviation since in the Loranthaceae they are generally triradiate. The pollen grain has a thick, smooth exine and a thin intine, and is 2-celled at the time of shedding.

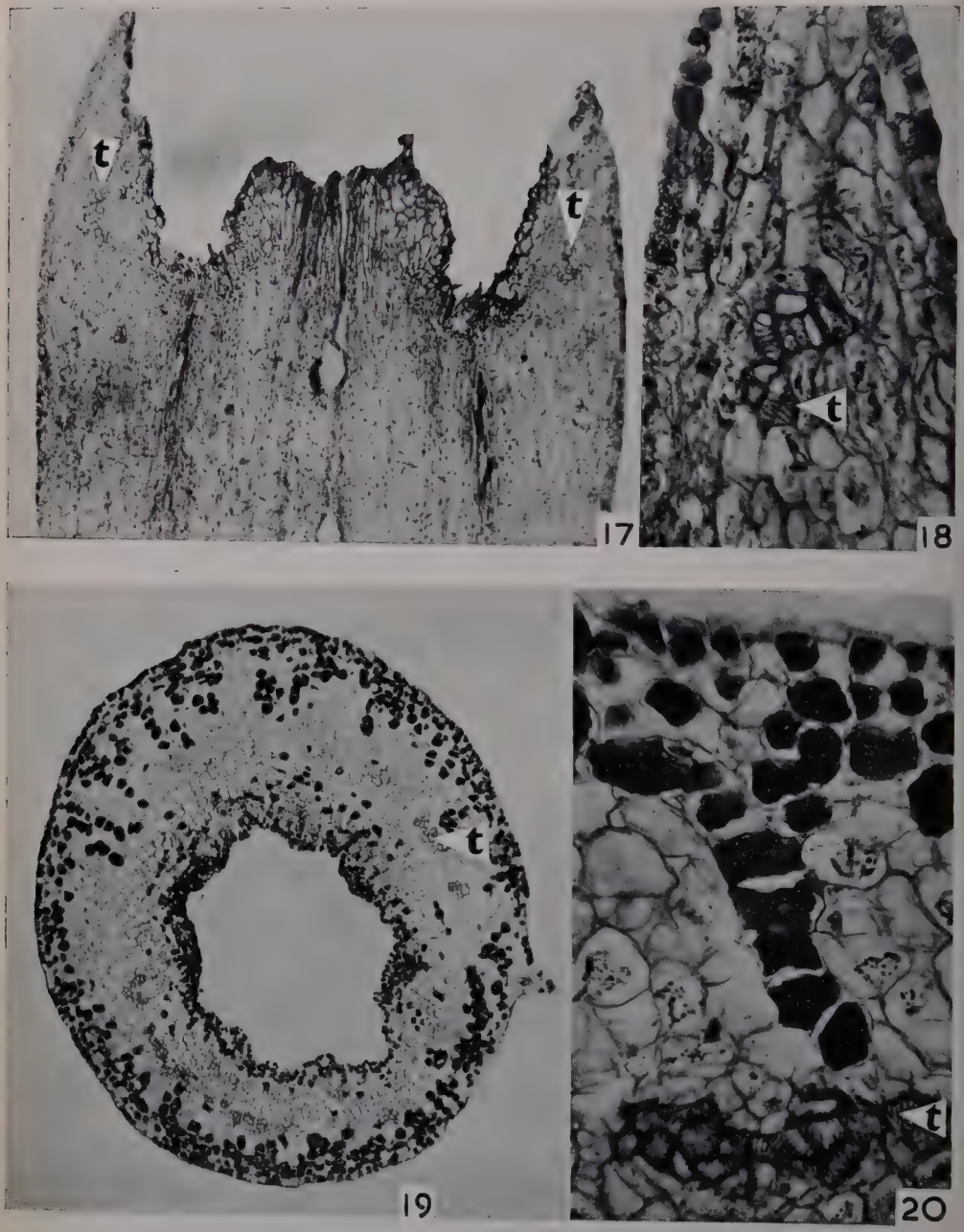
FEMALE GAMETOPHYTE — The earliest stage of the female gametophyte observed in the material available to me is a 4-nucleate embryo sac (Fig. 24). As in *Macrosolen* (Maheshwari & Singh, 1952), *Lysiana* (Narayana, 1958a) and *Lepeostegeres* (Dixit, 1958), a 6-nucleate condition (Fig. 26) precedes the 8-nucleate stage. The four nuclei at the lower end organize into three antipodal cells and the lower polar nucleus. The lower end of the embryo sac extends downwards leaving the antipodal cells *in situ* (Figs. 26, 28, 30. 31, 32b). The two nuclei at the upper end remain a little behind the tip (Fig. 27) and of the four nuclei formed from their division, three organize into the egg apparatus and the fourth one functions as the upper polar nucleus (Figs. 28-30, 32a). The tips of the embryo sacs do not extend beyond the base of the style (Fig. 25) and often show a (sometimes two) short 'caecum' which is probably haustorial in function (Figs. 32a, 33-35). Rarely, the embryo sac may have a reversed polarity (Fig. 25, es_1).

The large synergids (Fig. 32a) degenerate early but their remnants persist even after the formation of the proembryo (Figs. 36, 39a). The polar nuclei fuse

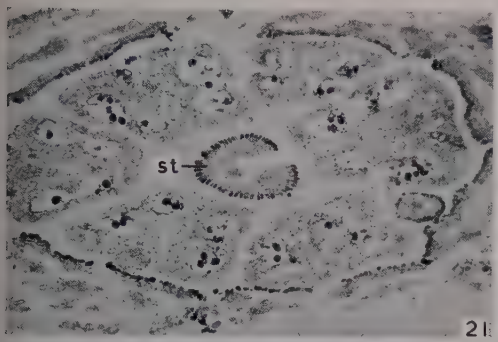


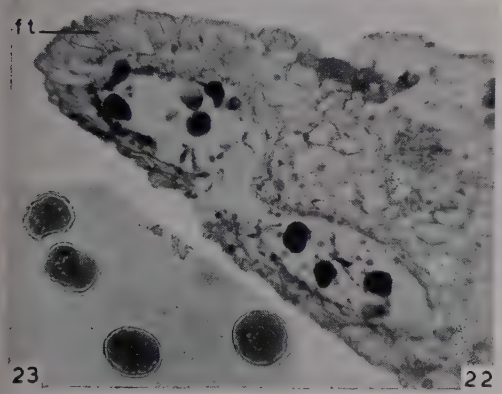
FIGS. 8-16 — (*ca*, calyculus; *ct*, collenchymatous tube; *es*, embryo sac; *sc*, stylar canal; *tr*, vascular tracheids). Fig. 8. L.s. bud. × 32. Figs. 9-16. T.s. bud at levels marked 9-16 in Fig. 8. × 96.

just below the egg apparatus (Fig. 32a). The three antipodal cells are usually arranged in a triradiate manner (Figs. 26, 28, 30, 31, 32b) and sometimes persist till the endosperm is fairly well advanced (Figs. 38, 39b).



FIGS. 17-20 — (*t*, vascular tracheids). Fig. 17. L.s. upper part of ovary showing vascular tracheids in each lobe of the calyculus. × 80. Fig. 18. Portion of calyculus enlarged from Fig. 17. × 200. Fig. 19. Calyculus in transection showing a discontinuous ring of tracheids. × 32. Fig. 20. Enlarged view of one group of tracheids. × 200.





FIGS. 21-23 — (*ft*, fibrous thickenings; *st*, style). Fig. 21. T.s. mature bud showing dehisced anthers. × 64. Fig. 22. Part of anther lobe enlarged to show fibrous thickenings in the endothecium. × 200. Fig. 23. Spherical pollen grains. × 405.

ENDOSPERM AND EMBRYO — The development of endosperm is similar to that reported in other members of the Loranthaceae. The earliest stage showed four cells arranged in two rows (Fig. 37). At first, due to repeated transverse divisions, the endosperm gradually extends upwards (Figs. 38, 39c). Subsequently periclinal divisions also occur resulting in a multiseriate arrangement. Endosperm develops simultaneously in several embryo sacs of the same ovary and during their growth the ovarian tissue in between them is consumed until they fuse to form a composite mass. With its further expansion the composite endosperm digests the adjoining parenchymatous tissue of the pericarp and then extends in between the carpellary vascular strands. This results in a deeply lobed condition (Fig. 7) and in transection the endosperm appears stellate (Fig. 47). The lobes also show branched or unbranched centripetal clefts running longitudinally as well as transversely (Figs, 42, 46). At its base the endosperm shows seven or eight lobes (Figs. 46, 48) which enclose the collenchymatous tube. Its apical portion is also correspondingly lobed (Figs. 46, 47).

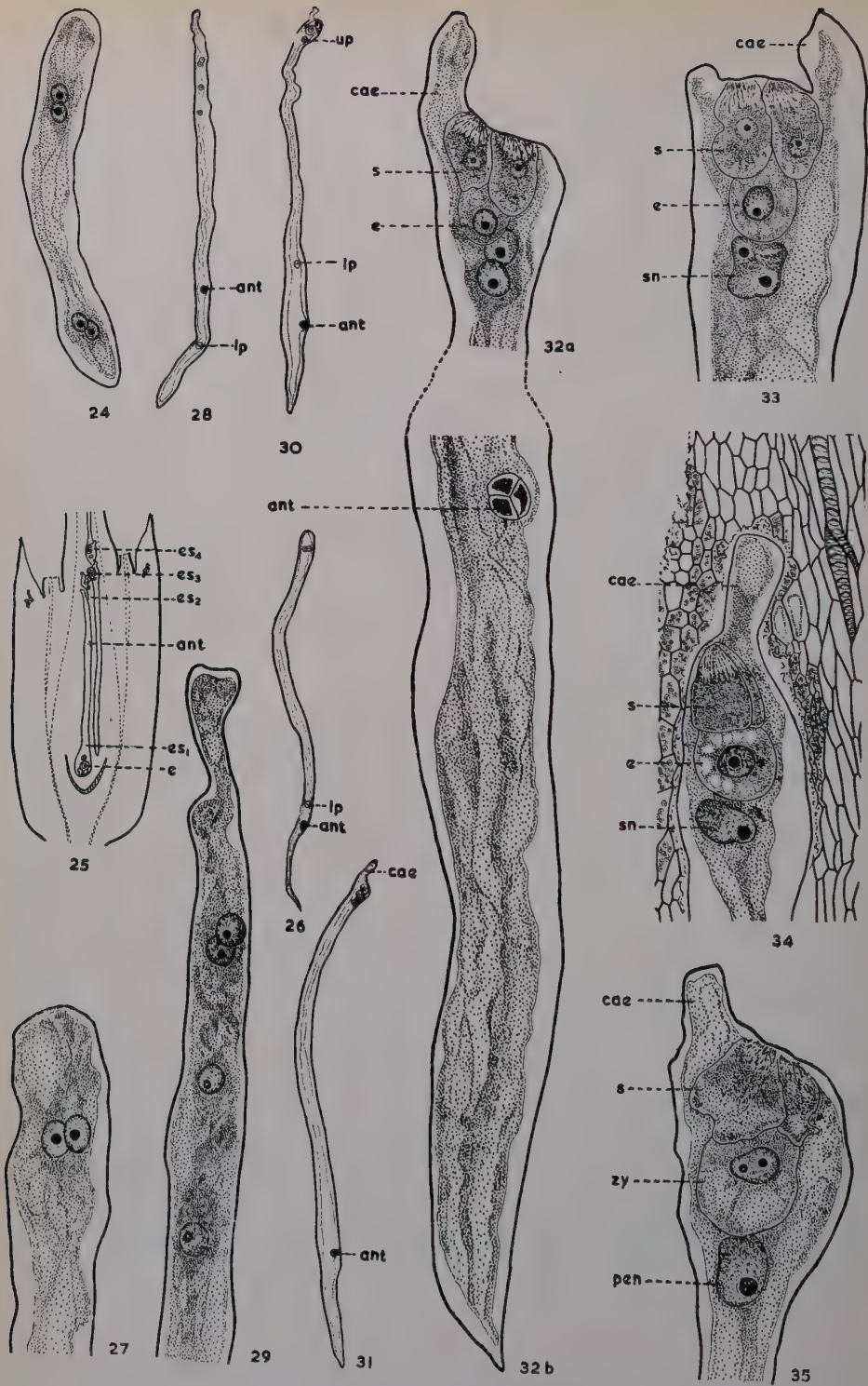
The zygote divides vertically and the 2-celled proembryo (Fig. 36) undergoes several transverse divisions (Figs. 38, 39a) giving rise to a biseriate proembryo. Due to rapid elongation of the suspensor the proembryo descends through the endosperm reaching up to the upper part of the collenchymatous tube (Fagerlind, 1959 calls it hypostase). At this time, due to repeated divisions, and elongation the suspensor becomes multiseriate and coiled (Figs. 49, 50). The coiling of the suspensor in the upper part of the fruit and rapid growth of the composite endosperm at its base are responsible for pushing the proembryo to a central position in the endosperm.

Generally several proembryos grow side by side but only one of them reaches down up to the collenchymatous tube and develops further.

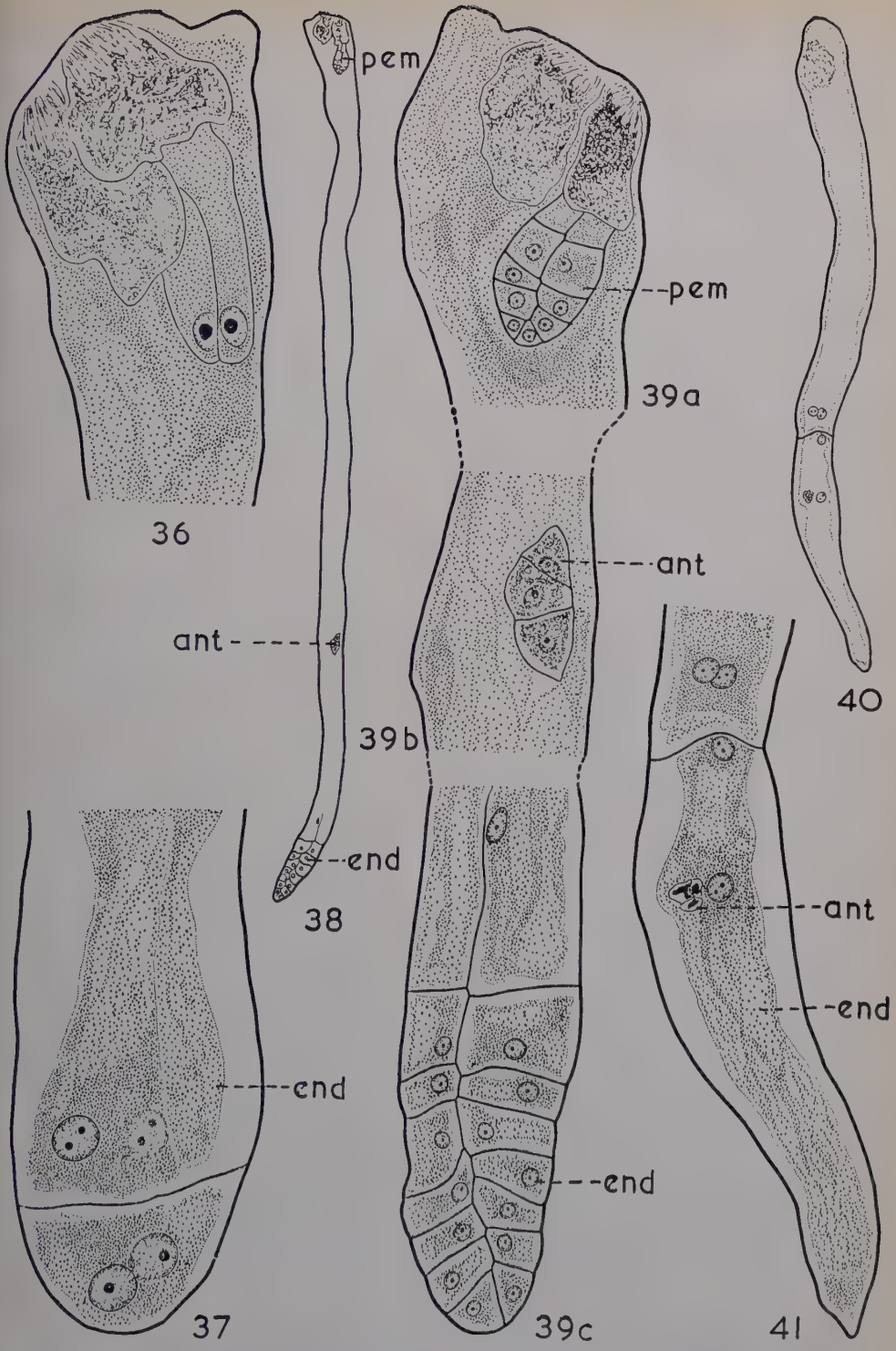
Occasionally, the proembryo may degenerate but endosperm formation proceeds normally (Figs. 40, 41).

FRUIT — The fruit consists of the pericarp, endosperm and embryo. The pericarp comprises the outermost leathery coat of thin-walled, parenchymatous cells interspersed with tannin-filled cells. It is followed by the viscid layer, several layers of sclerotic cells, and the vascular tissue (Fig. 44). The well developed stony zone is responsible for the drupaceous nature of the fruit (Fig. 45).

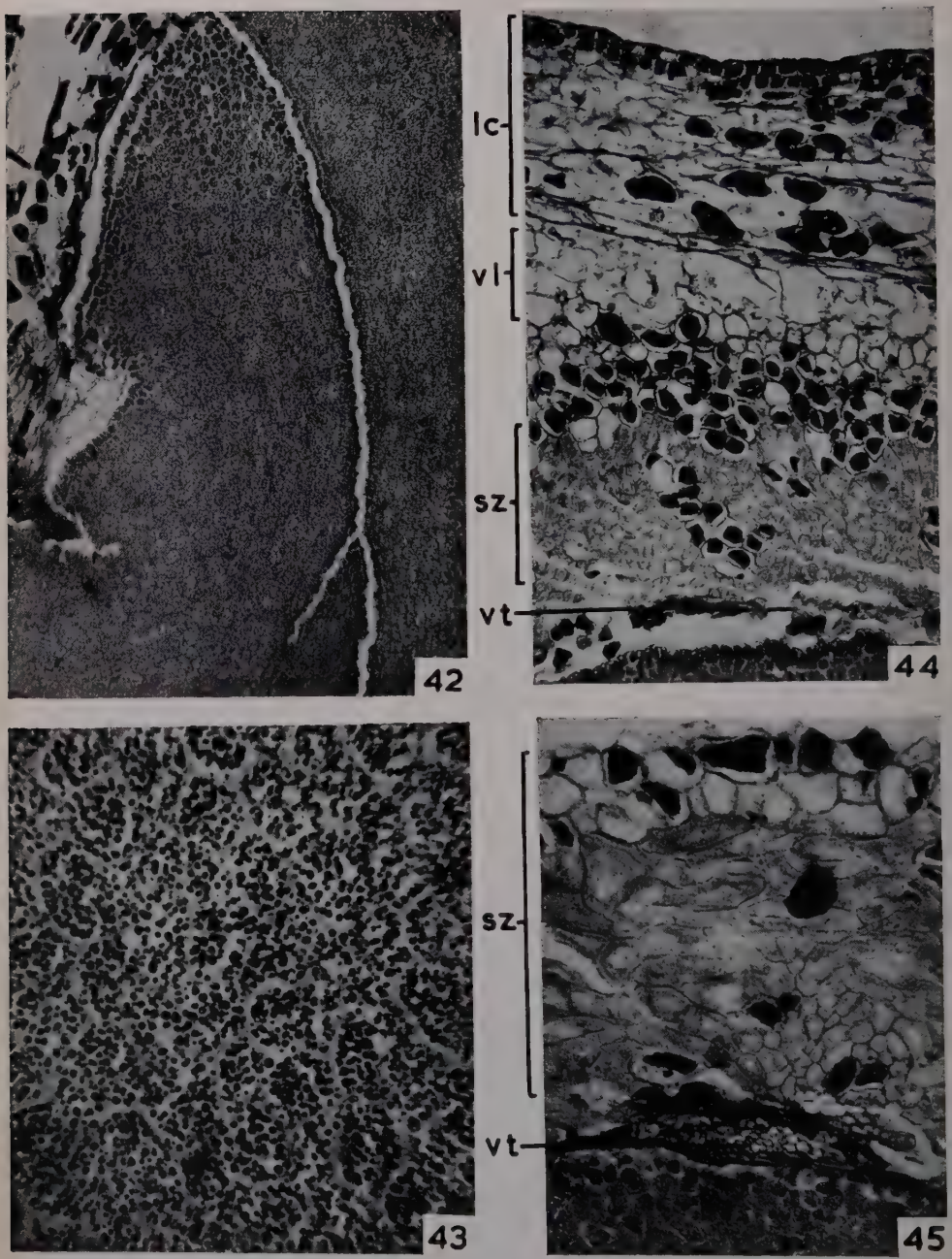
The mature endosperm is massive and encloses the embryo completely. The latter is typically dicotyledonous (Figs. 49, 50) and its radicular end does not project beyond the endosperm. The cotyledons remain free throughout their length (Figs. 49, 50). The cells of the endosperm and embryo are gorged with granular ovoid contents (Fig. 43). The outer tangential wall of the epidermal cells of endosperm is cutinized.



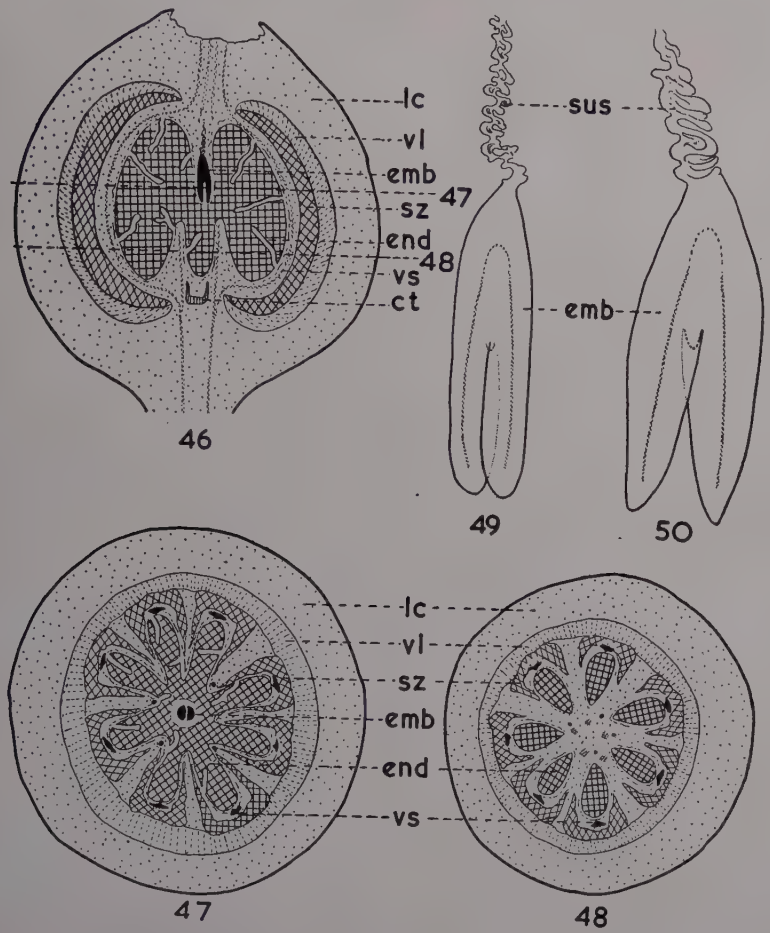
FIGS. 24-35 — Figs. 25 and 34 from microtome sections, rest from whole mounts. (*ant*, antipodal cells; *cae*, caecum; *e*, egg; *es*, embryo sac; *lp*, lower polar nucleus; *pen*, primary endosperm nucleus; *s*, synergid; *sn*, secondary nucleus; *up*, upper polar nucleus; *zy*, zygote). Fig. 24. Four-nucleate gametophyte. × 950. Fig. 25. L.s. ovary showing the extent of embryo sacs; note inverted polarity in es_1. × 96. Fig. 26. Six-nucleate gametophyte; the antipodal cells have already organized. × 194. Fig. 27. Enlargement of the tip of embryo sac from Fig. 26. × 50. Fig. 28. Eight-nucleate gametophyte. × 194. Fig. 29. Upper part of embryo sac from Fig. 28. × 950. Figs. 30, 31. Mature embryo sac. × 194. Fig. 32a, b. Enlargements of upper and lower portions of embryo sac from Fig. 31. × 950. Fig. 33. Tip of embryo sac showing egg apparatus and secondary nucleus. × 950. Fig. 34. Tip of embryo sac showing caecum in contact with the degenerated adjacent tissue. × 950. Fig. 35. Tip of fertilized embryo sac. × 950.



FIGS. 36-41 — All figures from whole mounts. (*ant*, antipodal cells; *end*, endosperm; *pem*, proembryo). Fig. 36. Two-celled proembryo. × 950. Fig. 37. Lower end of embryo sac showing 4-celled endosperm (one of the walls of each cell is in the plane of the paper). × 950. Fig. 38. Outline diagram for Fig. 39. × 194. Fig. 39a-c. Enlargements of upper, middle and lower portions of the embryo sac from Fig. 38. × 950. Fig. 40. Embryo sac showing 4-celled endosperm; the contents at the tip have degenerated. × 194. Fig. 41. Enlargement of endosperm from Fig. 40. × 400.



FIGS. 42-45 — (*lc*, leathery coat; *sz*, stony zone; *vl*, viscid layer; *vt*, vascular tissue). Fig. 42. Portion of endosperm showing the peripheral grooves. × 64. Fig. 43. Magnified view of endosperm cells. × 230. Fig. 44. L.s. pericarp; note the stony zone inner to the viscid layer. × 60. Fig. 45. Stony zone in transection. × 80.



FIGS. 46-50 — (*ct*, collenchymatous tube; *emb*, embryo; *end*, endosperm; *lc*, leathery coat; *sus*, suspensor; *sz*, stony zone; *vl*, viscid layer; *vs*, vascular supply). Fig. 46. L.s. mature fruit. × 12. Figs. 47, 48. T.s. at levels marked 47 and 48 in Fig. 46. × 12. Figs. 49, 50. Whole mounts of nearly mature and mature embryos. × 96.

Discussion

The morphological nature of the calyculus has been much discussed. Roxburgh (1874) and Haines (1924) considered it to be true calyx. Danser (1931) used the term calyx for the outer floral envelope "more on practical grounds". On the other hand, Eichler (1878) regarded calyculus as an axial structure. Engler (1888-1889) and Schaeppi & Steindl (1942) supported this view and emphasized that the calyculus is never calyx-like as the lobing of its margin, wherever present, is always irregular. Similar observations were made by Maheshwari & Singh (1952), and Singh (1952), and the former authors interpreted calyculus as an organ *sui generis*.

Narayana (1955, 1958b) demonstrated, for the first time, a distinct vascular supply in the calyculus of *Nuytsia floribunda* and suggested that calyculus should be regarded as true calyx. *Atkinsonia ligustrina* shows only isolated group of tracheids in each lobe of the calyculus, but these tracheids

are not connected with the main vascular supply (Figs. 8, 13). The feeble vasculature observed in this genus can be considered as a connecting link between the distinct vascular supply of *Nuytsia* and such members of the Loranthaceae where the calyculus is altogether devoid of a vascular supply. I have, therefore, preferred to designate the inner floral envelope as corolla instead of perianth as termed by previous authors.

Atkinsonia differs from the other Loranthaceae in possessing spherical pollen grains. The only other member of the family showing a similar condition is *Tupeia* (Van Tieghem, 1895; Danser, 1933; Smart, 1952).

The pericarp of Loranthaceae is generally distinguishable into four zones: the outer fleshy or leathery coat followed by the viscid layer, parenchymatous zone and a region of vascular tissue. In *Atkinsonia* an additional sclerotic zone inner to the viscid layer (Figs. 44, 45) is also present which is responsible for the drupaceous nature of the fruit.

Summary and Conclusions

The vascular anatomy of the flower indicates that the gynoecium is tetracarpellary.

The anther wall consists of the epidermis, fibrous endothecium, two middle layers and the glandular tapetum. The mature pollen grains are spherical and 2-celled.

A 6-nucleate condition precedes the 8-nucleate stage during the development of the female gametophyte. Five or six embryo sacs develop simultaneously in an ovary and their extension is limited up to the base of the style. Besides the long chalazal caecum, a short caecum also develops from the tip of the embryo sac.

The primary endosperm nucleus travels to the base of the embryo sac situated near the collenchymatous tube.

The endosperm is cellular and develops from the base of the embryo sac upwards. Concurrent development of endosperm in several embryo sacs in an ovary eventually leads to a composite mass. The mature endosperm is deeply lobed and appears stellate in a cross-section.

The zygote divides vertically and subsequent divisions are transverse resulting in a biseriate proembryo. The terminal and subterminal proembryonal tiers give rise to a mass of cells from which differentiates the embryo proper. The mature embryo is dicotyledonous and the long coiled suspensor can be recognized even at this stage.

The fruit is a pseudodrupe. The seed is 'naked', and the pericarp shows a sclerotic zone inner to the viscid layer.

Danser (1933) assigned *Atkinsonia*, together with *Gaiadendron*, to the subtribe Gaiadendrinae under the tribe Elytrantheae. Since *Atkinsonia* possesses dorsifixed versatile anthers, deeply lobed endosperm and drupaceous fruit — features typical of the subtribe Gaiadendrinae, its placement in this subtribe is fully justified. The genus is peculiar in having spherical pollen grains and vascular tracheids in the lobes of the calyculus.

An embryological study of *Gaiadendron* is likely to be very useful in distinguishing Gaiadendrinae from Elytranthinae. In Danser's (1933) classification subtribe Elytranthinae precedes Gaiadendrinae. Since *Atkinsonia* is a root parasite, and shows spherical pollen grains and a drupaceous fruit, it is suggested that Gaiadendrinae be placed before Elytranthinae in the tribe Elytrantheae (*see* also Engler & Prantl, 1897). Its primitivity is further evident by the presence of vasculature in the calyculus.

I am grateful to Dr. B. M. Johri and Professor P. Maheshwari for guidance and useful comments. Thanks are also due to the Ministry of Education for awarding a Senior Research Training Fellowship.

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*Not seen in original.

MORPHOLOGICAL AND EMBRYOLOGICAL STUDIES IN THE FAMILY LORANTHACEAE — VIII. *TOLYPANTHUS* BL.

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The literature on the embryology of the subfamily Loranthoideae has already been reviewed by Johri, Agarwal & Garg (1957). There is practically no work on the embryology of *Tolypanthus* except for a short note published by me (Dixit, 1956). I have studied two species, *T. lagenifer* (Wight) Van Tiegh. and *T. involucratus* (Roxb.) Van Tiegh.[1], and the following description refers mainly to the first species while some noteworthy features of *T. involucratus* have also been included.

Material and Methods

The material of *T. lagenifer* was obtained through the courtesy of Dr R. D. Adatia, Rev. Father H. Santapau

1. The following are the synonyms (*see* Danser, 1929, 1933):
(i) *Tolypanthus lagenifer* (Wight) Van Tiegh.= *Loranthus lageniferus* Wight, *L. lageniflorus* Hook., *Tolypanthus lageniferus* Van Tiegh.
(ii) *T. involucratus* (Roxb.) Van Tiegh.= *Elytranthe gemmiflora* G. Don., *Loranthus involucratus* Roxb.

*This work was carried out at the Department of Botany, University of Delhi, Delhi 6.

(Bombay), Professor T.S. Mahabale (Poona) and Dr A. Nagaraja Rao (Bangalore), and of *T. involucratus* from Dr V. Raghavan (Gauhati). During August 1955, I also collected *T. lagenifer* from Khandala (Bombay). Formalin-acetic-alcohol and Nawaschin's fluid were used for fixation and usual methods of dehydration and imbedding were followed. Sections were cut 4-20 microns thick and stained with safranin and fast green.

Observations

External Morphology — The subsessile flowers of *Tolypanthus lagenifer* are enclosed in a 5- or 6-toothed, red or pink cupule or involucre (Figs. 1-3), whereas in *T. involucratus* the flowers are borne on four, large, foliaceous bracts (Figs. 6-9). The calyculus (calyx)[2] which, is more prominent in the latter species, forms a tube around the base of the corolla[3]. In an open flower the tips of the corolla segments become strongly reflexed (Figs. 4, 9-11). The anthers are oblong-linear (Figs. 5, 11), the ovary is ovate-oblong and has a long style terminating in a capitate stigma (Figs. 10, 11), and the fruit is a " pseudo-berry " crowned by the persistent cylindrical calyculus and a short stub of the stylar base (Fig. 43).

Vascular Supply — In both the species the vascular supply of the flower is based on a pentamerous plan and resembles that of *Amyema* (Dixit, 1958a).

The base of the flower shows a ring of five vascular bundles which divide and redivide until a pentangular ring of 20 to 24 bundles is formed. At a slightly higher level three or four bundles fuse at each corner of the ring while the remaining bundles rearrange into an inner ring. The bundles of the outer ring enter the adnate portion of the corolla and filament while in their free portions each corolla lobe as well as the filament receives a single trace. The bundles of the inner ring enter the style and reach up to the stigma where they branch. The calyculus does not show any vascular supply.

Organogeny — The floral organs arise in acropetal succession (Figs. 12-15). The calyculus differentiates as a small rim followed by the primordia of corolla and androecium. As the stamens elongate, the primordia of gynoecium become distinguishable.

During the earlier stages of development the narrow stylar canal is continuous with the broad ovarian cavity (Figs. 13-17) and a conical ' mamelon ' develops from its base (Figs. 18-21).

Microsporogenesis and Male Gametophyte — There is a high percentage of sterility in the pollen of *T. lagenifer*, and the study of microsporogenesis and male gametophyte is mainly based on the material of *T. involucratus*.

The wall of a young anther consists of the flattened epidermis, fibrous endothecium, a single middle layer and glandular tapetum. The middle layer collapses during enlargement of the microspores. The tapetum, which becomes 2-layered at places, is absorbed by the maturing pollen grains. The reduction divisions are

2. Narayana (1958) observed vascular tissue in the calyculus of *Nuytsia floribunda* and interpreted this structure as calyx. Therefore, perianth should be designated as corolla and I have preferred the use of the latter term.

3. Fagerlind (1959) regards the collenchymatous pad as hypostase and I have also adopted this terminology.

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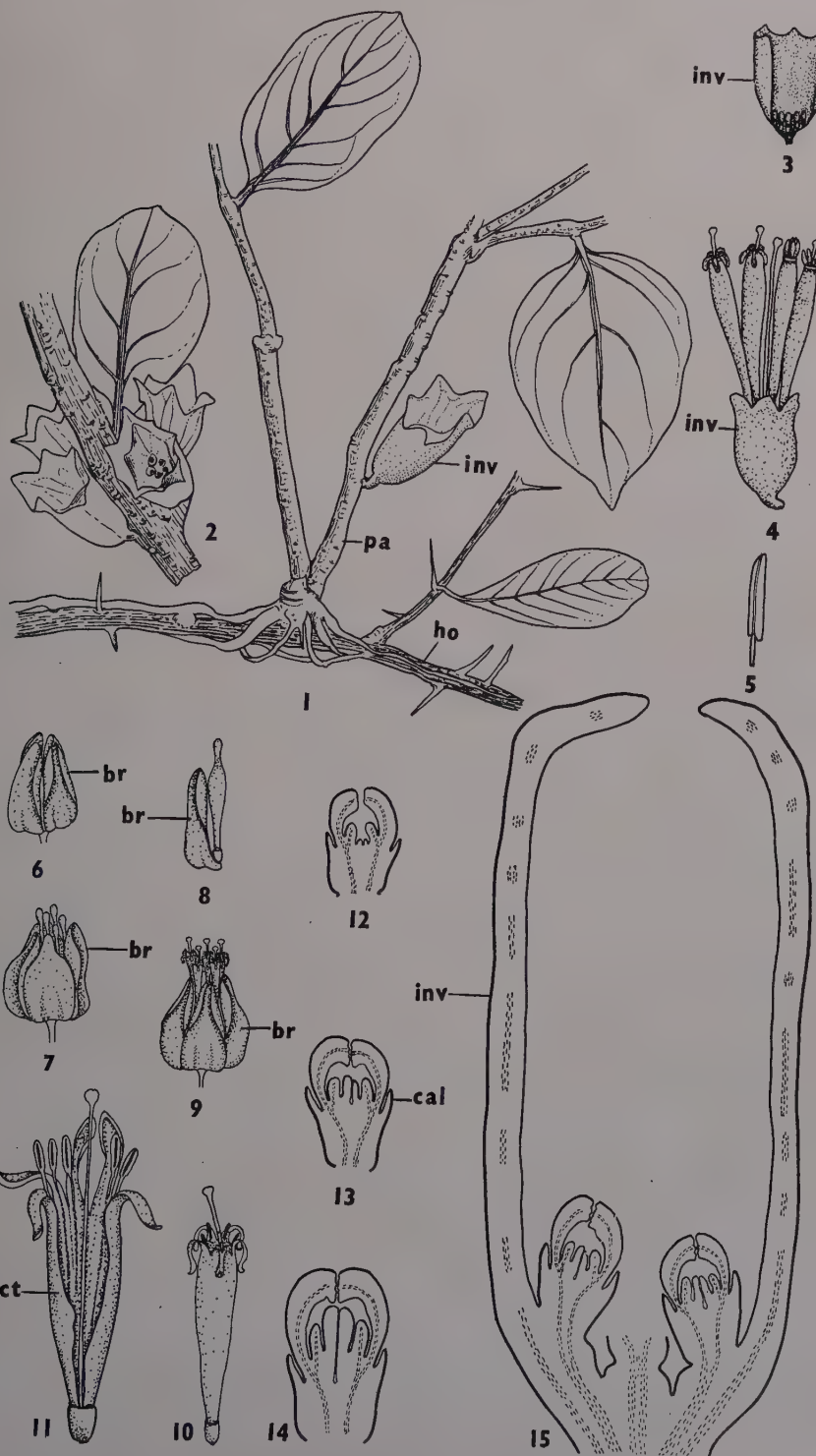
Figs. 1-15 — External morphology. (*br*, bract; *cal*, calyculus; *ct*, corolla tube; *ho*, host; *inv*, involucre; *pa*, parasite). Figs. 1-5, 12-15. *Tolypanthus lagenifer*. Fig. 1. Flower-bearing twig attached to the branch of host (*Randia brandissi* Gamble). × ¾. Fig. 2. Portion of stem with four inflorescences. × ¾. Fig. 3. Involucre cut open to show young buds. × 2. Fig. 4. Involucre bearing open flowers. × 2. Fig. 5. Enlarged view of stamen. × 8. Figs. 6-11. *T. involucratus*. Fig. 6. Young inflorescence; note the four large foliaceous bracts. × 2. Fig. 7. Inflorescence containing mature buds. × 2. Fig. 8. Single bud attached to the bract. × 2. Fig. 9. Inflorescence showing open flowers. × 2. Fig. 10. Single flower. × 4. Fig. 11. Same, corolla tube split open to show the style. × 6. Figs. 12-14. L.s. buds showing progressive development of floral organs. × 60. Fig. 15. L.s. young inflorescence. × 60.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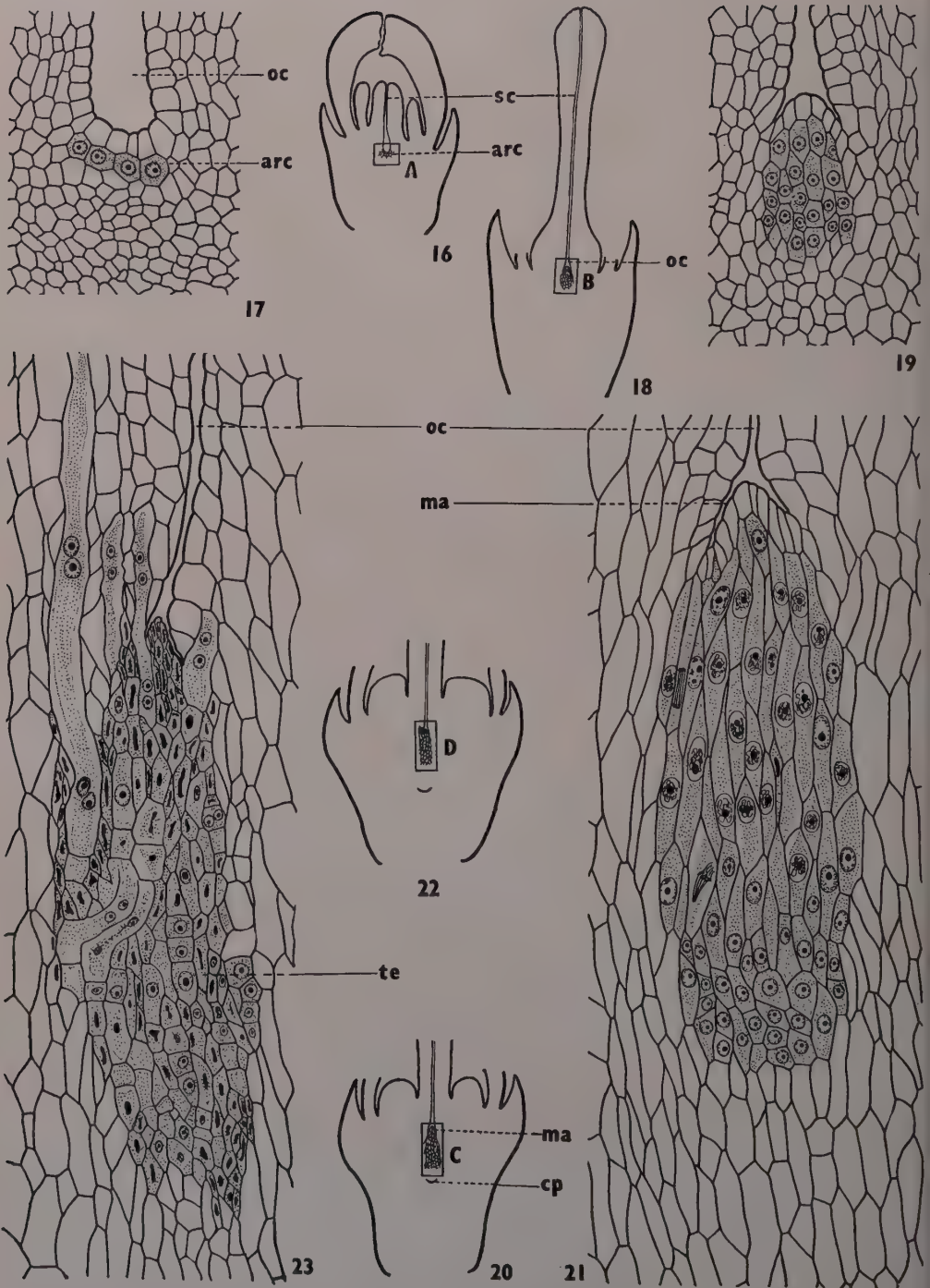
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simultaneous, cytokinesis takes place by furrowing and quadripartition is brought about by centripetal wedges of the special mucilaginous wall. Mostly tetrahedral, rarely decussate tetrads are formed.

The microspore nucleus shifts adjacent to the wall and divides resulting in a smaller generative and a larger vegetative cell. Subsequently, due to the dissolution of the separating membrane, the generative cell with its distinct cytoplasmic sheath moves closer to the vegetative nucleus. The pollen grains are tri-radiate with a thick and smooth exine, a thin intine, and apical germ pores. Dehiscence of the anther occurs by four longitudinal slits even before the opening of the flower.

MEGASPOROGENESIS — A multicelled archesporium differentiates in subepidermal layer at the base of the ovarian cavity (Figs. 16, 17). The epidermal cells lining the cavity often simulate the archesporial cells and contain dense cytoplasm and prominent nuclei. Repeated divisions of the archesporial cells result in a massive sporogenous tissue and at the same time floor of the ovarian cavity is pushed up into a conical mamelon (Figs. 18, 19). The sporogenous cells elongate and function as megaspore mother cells (Figs. 20, 21). Only a few of them undergo meiotic divisions resulting in linear tetrads (Figs. 22-27).

EMBRYO SAC — Figures 22 and 23 represent the development of the embryo sac in *T. lagenifer* and Figs. 24-33 in *T. involucratus*. One or more megaspores of the same tetrad may function and usually the uppermost elongates in the direction of the style (Figs. 22, 23). However, if the second or the third megaspore develops, at first it grows laterally but subsequently follows the longitudinal course. The nucleus migrates into this tubular extension and by two consecutive divisions forms 2- and 4-nucleate embryo sacs (Figs. 28, 29, 31). The upper end of the 4-nucleate gametophyte elongates further and extends into the stylar tissue. The tips of a number of embryo sacs showed two and four nuclei but their lower ends could not be traced. It appears that like *Amyema* (Dixit, 1958a) and *Lepeostegeres* (Dixit, 1958b), the two lower nuclei divide first and organize into three (uninucleate) or two (one uninucleate and the other binucleate) antipodal cells and the lower polar nucleus. Older embryo sacs showed a well developed caecum with the antipodal cells (which did not contain nuclei probably due to early degeneration) attached laterally. At first the two upper nuclei lie somewhat behind the tip but finally move up and divide. The daughter nuclei organize into the egg apparatus and upper polar nucleus (Fig. 33).

The tips of 10 to 12 embryo sacs ascend up to different lengths in the style (Fig. 32). In *T. lagenifer* the apical part of the longest embryo sac extends to more than half the length of 35-40 mm long style, while in *T. involucratus* it reaches up to two-thirds the length of the 15-17 mm long style. The length of the embryo sac from base to apex varies from 20 to 25 mm in *T. lagenifer* and 10 to 12 mm in *T. involucratus*. Due to their tortuous course and twisting around each other, it is difficult to trace the course of individual embryo sac.

FERTILIZATION—Pollination takes place in the closed bud and stigmatic papillae arrest the pollen grains. The pollen tube emerges through one of the three germ pores, makes its way along the stylar tissue until it comes to lie in the stylar canal adjacent to the gametophyte. The tip of the pollen tube swells and occasionally shows a tendency to branch. The entry of the tube in the embryo sac and the events leading to fertilization were

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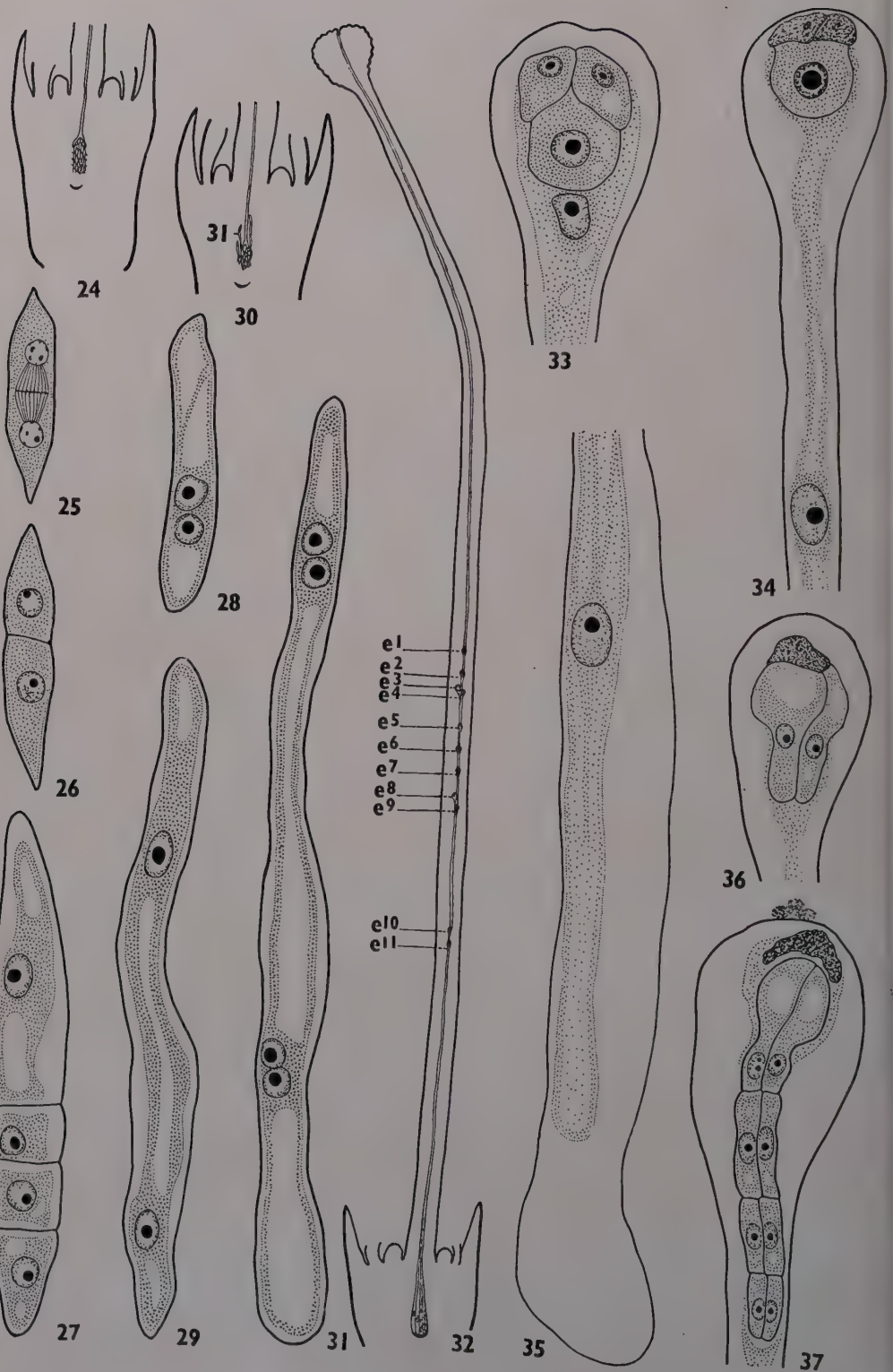
FIGS. 16-23 — *Tolypanthus lagenifer*. Megasporogenesis and female gametophyte. [*arc*, archesporium; *cp*, collenchymatous pad (hypostase); *ma*, mamelon; *oc*, ovarian cavity; *sc*, stylar canal; *te*, tetrad]. Figs. 16, 18, 20. Outline diagrams of Figs. 17, 19 and 21 respectively. × 95. Fig. 17. Portion marked *A* in Fig. 16 showing subepidermal archesporium. × 485. Fig. 19. Mamelon with adjacent ovarian tissue marked B in Fig. 18. × 970. Fig. 21. Region marked C in Fig. 20, showing megaspore mother cells. × 970. Fig. 22. Outline diagram of Fig. 23. Fig. 23. Portion marked D in Fig. 22, showing tetrads, and 2- and 4-nucleate embryo sacs. × 970.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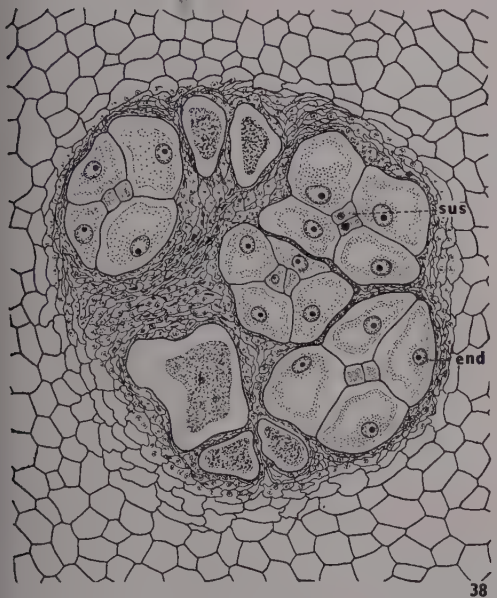

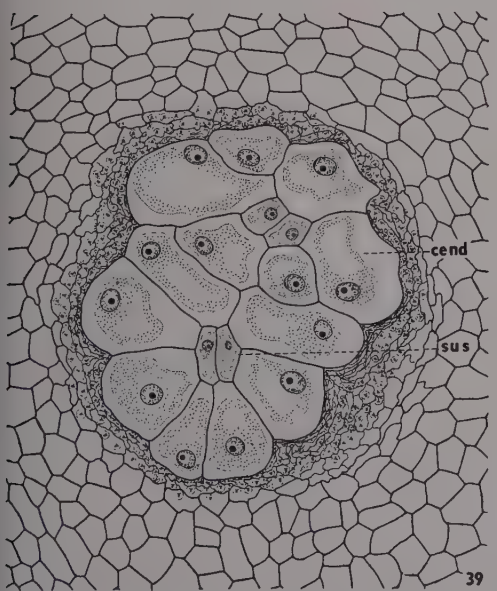

not observed. However, from the later stages it is inferred that double fertilization takes places normally. The remnants of the pollen tube persist even after the formation of the proembryo.

ENDOSPERM AND EMBRYO — After fertilization the primary endosperm nucleus travels to the lower part of the embryo sac situated in the ovary (Figs. 34, 35). The endosperm is of the cellular type and at an early stage the cells show a 4-rowed arrangement (Fig. 38). Figures 38 and 39 represent stages in the formation of composite endosperm. The endosperm cells enlarge rapidly and become vacuolate but those adjacent to the hypostase (collenchymatous pad) remain smaller and richly cytoplasmic.

Meanwhile, the zygote enlarges, elongates downwards and divides vertically (Fig. 36). This is followed by transverse divisions resulting in a long, biseriate proembryo (Fig. 37). Further divisions and elongation of the suspensor cells gradually push the proembryo into the basal part of the embryo sac. It grows through the endosperm as far as the hypostase (Figs. 40, 41).

Several proembryos develop concurrently in the same style. Two or three of these reach the ovary but only one grows vigorously while the others abort. The suspensor, which is biseriate in the stylar

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FIGS. 38, 39 — *Tolypanthus involucratus*. Endosperm and embryo. (*cend*, composite endosperm; *end*, endosperm; *sus*, suspensor). Fig. 38. T.s. ovary showing four embryo sacs containing 4-seriate endosperm and biseriate suspensor; remnants of five other degenerated embryo sacs are also seen. × 970. Fig. 39. Same, showing composite endosperm and two biseriate suspensors. × 970.

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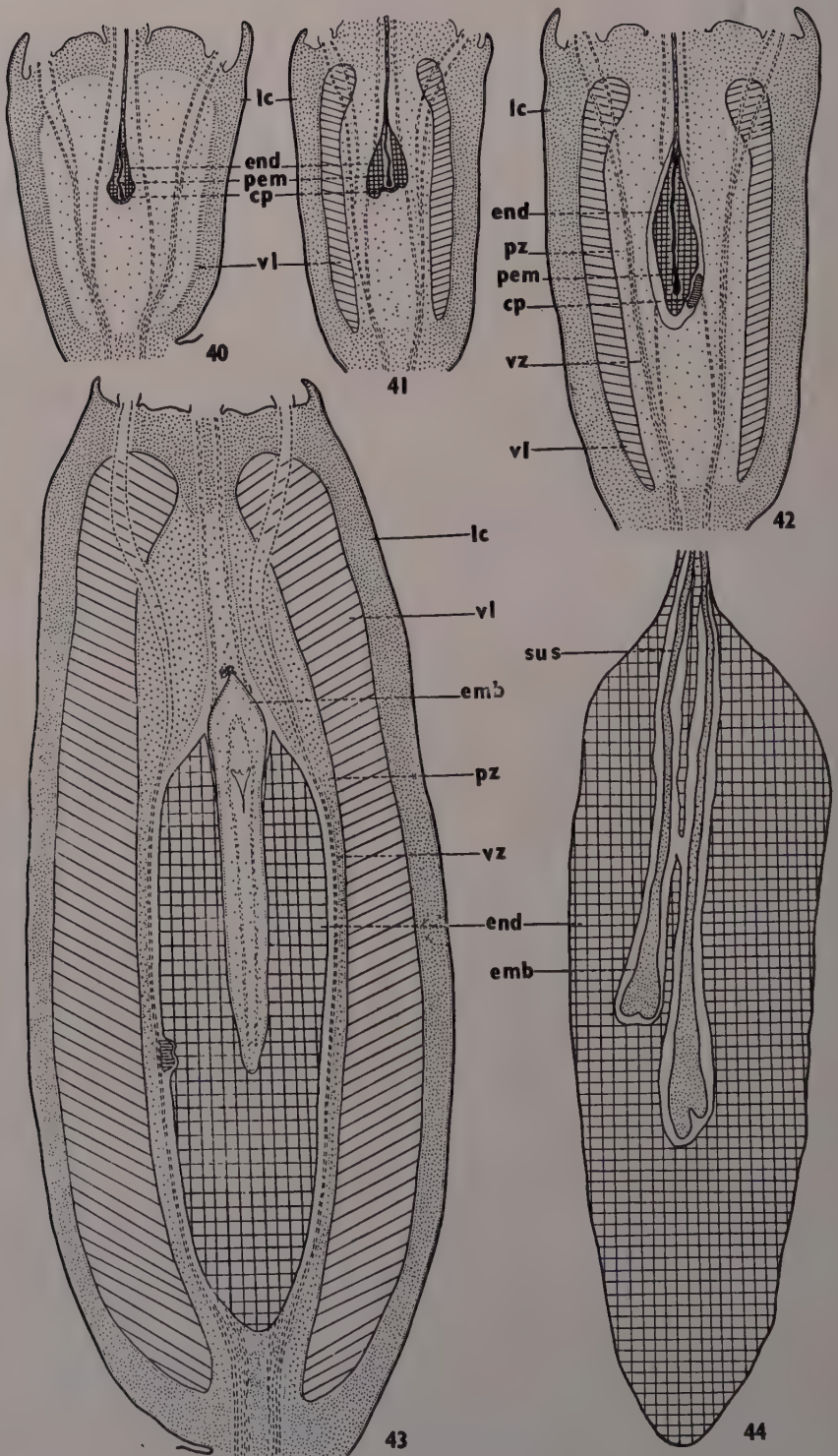
FIGS. 24-37 — Figs. 24-33, 37 of *Tolypanthus involucratus* and Figs. 34-36 of *T. lagenifer*. Female gametophyte and proembryo. Figs. 24, 30. Outline diagrams of Figs. 25 and 31 respectively. × 32. Fig. 25. Megaspore mother cell from Fig. 24. × 1820. Fig. 26. Dyad. × 1820. Fig. 27. Linear tetrad; the uppermost megaspore is functional. × 1820. Figs. 28, 29. 2-nucleate embryo sacs. × 1820. Fig. 31. 4-nucleate embryo sac marked in Fig. 30. × 1820. Fig. 32. Diagrammatic representation of a carpel showing tips of 11 embryo sacs in the style. × 32. Fig. 33. Upper end of embryo sac with egg apparatus and upper polar nucleus. × 970. Fig. 34. Same, showing degenerated synergids, zygote and primary endosperm nucleus. × 970. Fig. 35. Part of caecum, situated in the ovary, containing primary endosperm nucleus. × 970. Fig. 36. Two-celled proembryo. × 970. Fig. 37. Young biseriate proembryo.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region, undergoes longitudinal divisions and becomes broader.

Henceforth, the development of endosperm and proembryo proceeds together. The composite endosperm grows around the hypostase and forms an annular bulge. Subsequent growth is restricted to only one side so that the hypostase (collenchymatous pad) is left laterally wedged into the endosperm (Figs. 40-43). Due to the lateral and basal extension of the endosperm the adjacent ovarian tissue (inner to the vascular zone) is crushed so that the endosperm comes in direct contact with the vascular strands which check its further growth (Figs. 42, 43). When the proembryo is at the heart-shaped or slightly older stage, starch grains appear in the endosperm and by the time the cotyledons differentiate the cells are packed with reserve food.

The embryonal cells divide actively leading to the formation of globular and heart-shaped stages (Figs. 41, 42, 44). The coiling and twisting of the suspensor pulls up the proembryo to a central position in the endosperm. Gradually the cotyledons differentiate and one of them invariably remains smaller. In a mature embryo the cotyledons become adpressed to each other and appear fused except in the region of the plumule (Fig. 43). However, the vascular supply of both the cotyledons remains quite distinct.

Twin embryos are quite common and Fig. 44 shows one embryo at the heart-shaped and the other at the early dicotyledenous stage. Occasionally, three cotyledons develop in an embryo and they also appear fused as in a normal embryo.

FRUIT — The fruit tapers below, gradually widens towards the middle and again becomes narrow at the apex. The pseudo-monocotyledonous embryo is enclosed in the endosperm except for the massive radicular end which projects beyond its apex (Fig. 43). In one instance, the radicular ends of two embryos were protruding laterally (sub-terminal) from the endosperm.

The fruit wall is distinguishable into an outermost leathery coat (Figs. 45-48) comprising the epidermis, one or two hypodermal layers, and seven to twelve layers of elongated parenchymatous cells. Some of the cells contain tannin which gives a reddish-brown tinge to the fruit. Unlike *Amyema* (Dixit, 1958a), this zone is very narrow and is devoid of stone cells (Figs. 45-48). Next to the leathery coat is the conspicuous viscid layer consisting of diagonally oriented, thin-walled cells filled with viscin. In the apical region of the fruit the viscid layer appears five-lobed. Then follows the parenchymatous zone which is considerably broader in the upper and lower portions of the fruit, and consists of elongated and highly vacuolate cells. The vascular strands run through the parenchymatous zone and the parencymatous cells inner to it are crushed by the endosperm. In the basal region of the fruit some of the cells develop into sclereids.

At the base of the fruit the vascular bundles are arranged in a ring. Slightly above the middle level of the endosperm, they become arranged into two rings — the outer one represents the traces to the corolla and androecium and the inner to the style and stigma.

Summary

The flowers of *Tolypanthus lagenifer* and *T. involucratus* are subsessile and pentamerous. In the former species they are enclosed in a 5- or 6-toothed cupule whereas in *T. involucratus* the flowers are borne on four, large, foliaceous bracts. The floral organs arise in acropetal succession: calyx, corolla, androecium and gynoecium. The mamelon is formed secondarily due to the

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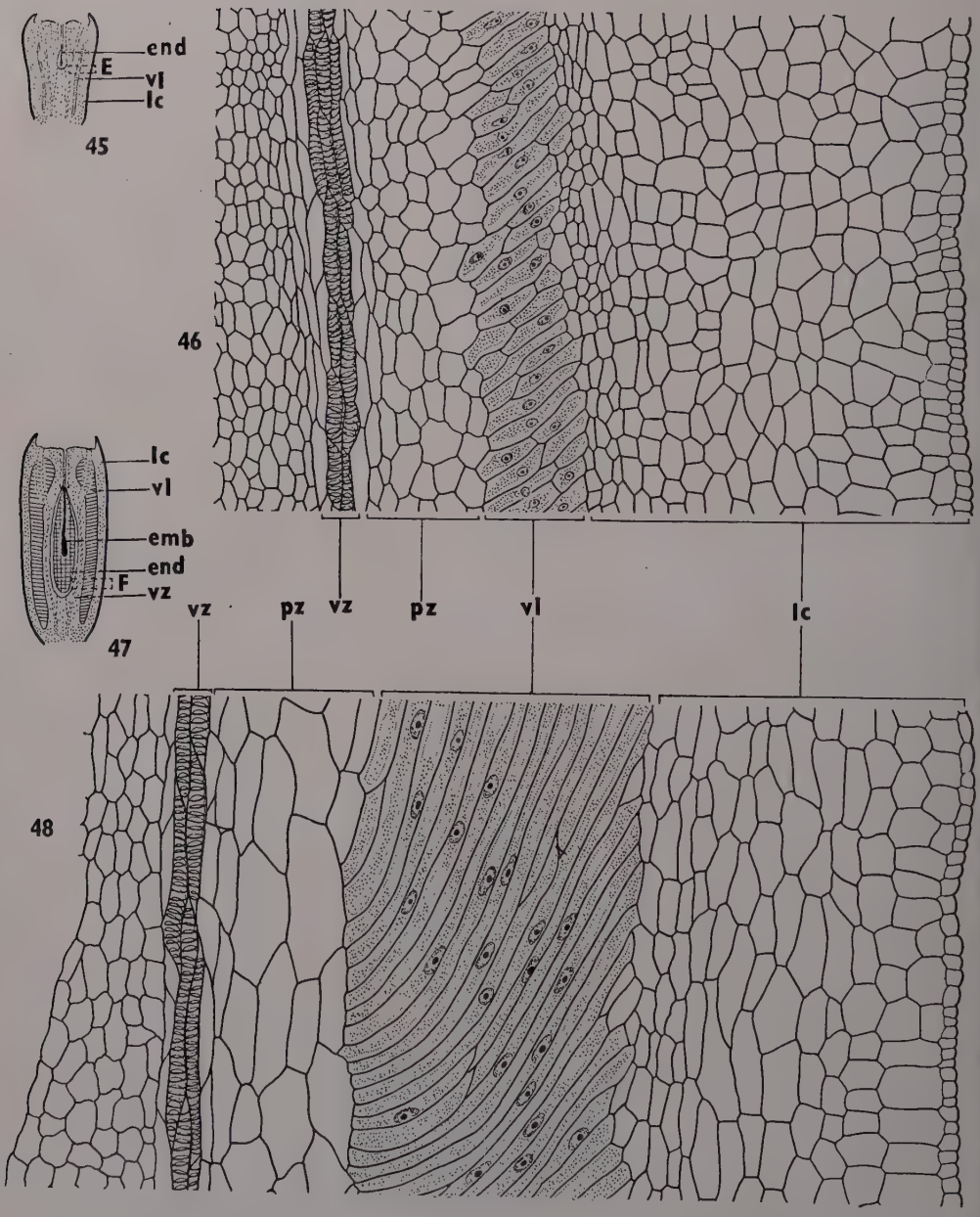
FIGS. 40-44 — *Tolypanthus lagenifer*. Endosperm and embryo. (*cp*, collenchymatous pad; *emb*, embryo; *end*, endosperm; *lc*, leathery coat; *pem*, proembryo; *pz*, parenchymatous zone; *sus*, suspensor; *vl*, viscid layer; *vz*, vascular zone). Fig. 40. L.s. young fruit showing terminal part of proembryo surrounded by composite endosperm; note the differentiation of viscid layer. × 32. Fig. 41. Same, older stage; pericarp has differentiated into three zones. × 32. Fig. 42. Still older stage of embryo; the collenchymatous pad has been displaced laterally. Fig. 43. Longisection of mature fruit. Fig. 44. L.s. composite endosperm with two embryos. × 95.



FIGS. 45-48 — *Tolypanthus lagenifer*. Pericarp. (*emb*, embryo; *end*, endosperm; *lc*, leathery coat; *pz*, parenchymatous zone; *vl*, viscid layer; *vz*, vascular zone). Figs. 45, 47. L.s. young and old fruits (diagrammatic). × 21. Figs. 46, 48. Portions of pericarp marked *E* and *F* in Figs. 45 and 47 respectively. × 420.

uplifting of the base of the ovarian cavity after the floral organs have fully developed.

The anther wall consists of the epidermis, fibrous endothecium, middle layer and glandular tapetum. The microspore

mother cells undergo simultaneous reduction divisions, and form tetrahedral and decussate tetrads. The pollen grains are triradiate and are shed at the 2-celled stage.

The female archesporium differentiates in the hypodermal layer at the base of the ovarian cavity. It divides repeatedly producing numerous sporogenous cells which directly function as megaspore mother cells. Megaspores of several linear tetrads and often more than one megaspore of the same tetrad develop concurrently and give rise to 10 to 12 embryo sacs which grow up to two-thirds the length of the style. The development of the embryo sac is of the Polygonum type.

A composite endosperm is formed as is common to other members of the family.

The zygote divides vertically followed by many transverse divisions producing a biseriate proembryo. Due to elongation of the suspensor the proembryo descends to the basal portion of the embryo sac containing endosperm. Further development of the embryo occurs here. Several embryos develop concurrently in the style but usually one, sometimes two, reach maturity. The fruit is a pseudoberry. The endosperm is directly surrounded by the pericarp which is distinguishable into a leathery coat, viscid layer and parenchymatous zone through which runs the vascular supply.

It gives me great pleasure to express my gratitude to Dr B. M. Johri and Professor P. Maheshwari of the Department of Botany, University of Delhi, for their guidance and help throughout the course of this work, and to Professor R. Misra, Department of Botany, Banaras Hindu University, for encouragement.

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A STUDY OF THE DEVELOPMENTAL POTENTIALITIES OF EXCISED LEAF PRIMORDIA IN STERILE CULTURE

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Introduction

The origin and development of leaves in vascular plants pose a number of intriguing problems for the student of morphogenesis. Leaves arise as primodia on the flanks of the apical cone, in an orderly sequence and in precise locations. At an early stage in its ontogeny, the leaf primordium, which initially very closely resembles the areas of meristem around it, begins to diverge in its characteristics from the meristem as a whole. Ordinarily it acquires a distinct dorsiventrality which, upon further development, leads to the characteristic flattening of the typical leaf. Its growth, moreover, appears to be accelerated above that of the surrounding meristem, and the leaf primordium soon overtops the apical meristem which produced it. Perhaps the most striking feature of leaf development, however, is the ultimate cessation of apical growth in all but a few exceptional cases and, after varying amounts of intercalary and lateral growth, the complete maturation of the organ. In other words, the leaf primordium is determinate in its destiny in contrast to the shoot apical meristem which, barring injury, inhibition or the onset of reproductive development, is indeterminate in its potentialities for growth.

An understanding of the causes underlying the divergence of the leaf primordium along its own morphogenetic pathway would seemingly be equivalent, except for details, to an understanding of morphogenesis itself; and to this challenge experimental morphologists have increasingly addressed themselves in recent years. Sussex (1951, 1955) was able to show, in a series of surgical experiments on potato, that the dorsiventrality of a leaf is caused by the activity of the shoot apical meristem and does not become fixed in a primordium simultaneously with the inception of its growth. Wardlaw (1949b, c) brought about the development of buds at presumptive leaf positions in *Dryopteris* by isolating them from the shoot apical meristem by tangential incisions. Subsequently, Cutter (1954, 1956), working with the same fern, induced the development of buds from the 3 youngest visible leaf primordia by isolating them from the apical meristem by wide, deep, tangential incisions, or by isolating them on plugs of tissue by four deep incisions. On the other hand, since buds can sometimes develop in direct continuity with an active apical meristem (Wardlaw, 1955a) and since dorsiventral leaves upon occasion arise from primordia isolated by deep incision from the meristem (Cutter, 1957), it must be concluded that the effect of the apical meristem, and in ferns, specifically the apical cell, upon leaf determination is excercised indirectly through the growth and organization of the shoot apex as a whole (Wardlaw & Cutter, 1955). The intriguing problem of the mechanism of leaf determination, therefore, although it has been more precisely defined by surgical experimentation, remains to be solved.

Concurrently with surgical studies on leaf development, another experimental approach to problems of leaf morphogenesis was being initiated, that of sterile nutrient culture of excised leaf primordia. It was found that isolated primordia of *Osmunda cinnamomea* L. and several other ferns could be grown to maturity in isolation from the parent plant on a medium of simple composition (Sussex & Steeves,

1953; Steeves & Sussex, 1957). Subsequently, this method was extended to the flowering plants with essentially similar results (Steeves *et al.*, 1957). It was, therefore, possible to conclude that, whatever the mechanism of leaf determination, it is a process which sets in motion a chain of morphogenetic events which is self-perpetuating. In other words, once a primordium has been determined, its subsequent development as a leaf is under the control of factors which are largely intrinsic rather than extrinsic. It must be pointed out that leaf development can be modified by external factors in such a way as to influence final size and shape (Sussex, 1958; Sussex & Clutter, 1960) and even the presence or absence of sporangia in certain ferns (Sussex & Steeves, 1958); but such factors produce modified leaves rather than some other category of plant organ.

These earlier experiments clearly dealt with primordia which had already been determined. In *O. cinnamomea*, primordia of a variety of sizes and degrees of development were cultured; but none younger than the tenth on the apex (P_{10}) were ordinarily excised. Because of the extremely slow rate of leaf development in this fern (Steeves & Briggs, 1958) the first 10 primoria are extremely small and poorly developed. It seemed reasonable to expect that at least some of these primordia might not be finally determined; and it was of interest to know what their developmental potentialities would be if grown in isolation from the shoot system. Accordingly an attempt was made to perfect a technique permitting the development of these minute masses of meristematic tissue. A preliminary account of some of these experiments has been published elsewhere (Steeves, 1957).

Materials and Methods

The major features of the methods used in the sterile culture of leaves of *O. cinnamomea* have been described in full recently (Steeves & Sussex, 1957) and need not be considered again here. Partially dissected apical buds were sterilized for 20 minutes in a 7 per cent by weight solution of Pittchlor[1] and washed in two changes of sterile water. No further sterilization was necessary since the interior of the bud is free of micro-organisms. Further dissection down to the youngest 10 primordia was carried out asceptically under a binocular dissecting microscope in a transfer room with the aid of alcohol-sterilized instruments. The removal of the primordia to be cultured was accomplished by means of fine knives fashioned by grinding from razor blade fragments and clamped directly into long-handled holders. In spite of the small size of the primordia, it has been possible to recognize their sequence with reasonable accuracy and to excise even the youngest (P_1) with very little injury. Excised primordia were transported on the knife directly to the medium in the culture vessels.

The basic nutrient medium used in these experiments consisted of a modified Knop's solution supplemented by trace elements as described in an earlier report (Steeves & Sussex, 1957), or of Knudson's solution (Knudson, 1925) together with the same trace elements and, in both cases, two per cent sucrose. The medium was solidified with 0·8 per cent agar. This medium was supplemented in certain experiments by the addition of the following constituents individually or in combinations: a mixture of B vitamins (Steeves & Sussex, 1957), acid casein hydrolysate, naphthalene acetic acid and both autoclaved and unautoclaved coconut milk. In addition, the sucrose concentration was varied from 0·5 per cent to 6·0 per cent in certain instances. In all cases the initial pH of the medium was adjusted to 5·5.

The primordia were grown in a variety of culture vessels, and their successful development was profoundly influenced by the nature of the container used. In early experiments standard test tubes of 25 mm or 16 mm diameter were used; but the survival of primordia in these was extremely poor. Subsequently, smaller tubes (11 mm diameter, 50 mm length) were tried with no improvement in survival rate. Excellent survival and growth

1. A commercial product containing 70 per cent calcium hypochlorite, manufactured by the Columbia-Southern Chemical Co., Pittsburgh, Pa.

were obtained, however, in Petri dishes of 60 mm diameter or in one ounce square bottles with a round mouth of a 33 mm diameter which was covered by a sheet of polyethylene film. Since, in spite of sealing with cellophane tape, the medium in the Petri dishes tended to dry out in less than the duration of an ordinary experiment, the bottles proved to be the more satisfactory vessels. The reasons for improved survival in certain culture vessels are not yet completely understood; but some of the factors involved are being considered elsewhere[2]. The primordia were grown in a culture room at a temperature of 24°C $\pm$ 1° in a daylength of 12 hours.

Primordia which were to be examined histologically were, for the most part, embedded in the desired orientation in agar and then fixed, together with an easily visible block of surrounding agar, in formalin-acetic acid-alcohol. They were subsequently dehydrated, embedded in paraffin, sectioned at 10 microns and stained with Heidenhain's iron alum haematoxylin with a counterstain of safranin, according to standard procedures.

Experimemtal Results

The first 10 visible leaf primordia of *O. cinnamomea* are all within the group of leaves previously designated as Set I (Steeves & Sussex, 1957). Under normal conditions these primordia would not be expected to complete their development for from 3 to nearly 4 years, depending upon whether they were destined to become cataphylls or fronds. The small size of these primordia, together with their close packing in the apical bud, makes their accurate recognition extremely difficult. Only by a careful dissection of the outer portions of the bud, so that the phyllotactic system is clear before the youngest primordia are reached, is it possible to identify these primordia with confidence.

The youngest primordium (P_1) has the form of a flat mound, with poorly defined limits, projecting a maximum of 30 microns from the surface of the meristem at its highest point (Fig. 1). The second (P_2) is similar, but projects slightly more and has more sharply defined limits. In excising these primordia it was ordinarily necessary to remove the primordium on a shallow plate of tissue of roughly the same outline as that of the primordium. All older primordia, however, could be cut off at the surface of the apical cone so that only the primordium was removed. Almost from its emergence as a mound, the primordium seems to be tipped towards the center of the apical mound. By the P_3 stage it shows a distinct curvature towards the center of the apex so that its own apex is directed 45 degrees or more from the vertical axis (Steeves & Briggs, 1958). Third primordia averaged approximately 280 microns in total length, but measurement was not accurate because of the curvature of the primordium. A progressive increase in size was noted in successively older primordia. The fifth (P_5) averaged approximately 450 microns in length and the 10th (P_{10}) slightly over 800 microns.

The leaf of *O. cinnamomea*, in a manner typical for ferns, is characterized by long-continued apical growth, and the apex of the leaf, at least while it is actively growing, possesses a tetrahedral apical cell. In considering the developmental potentialities of excised leaf primordia, it was important to know at what stage the apical cell is set off, since in *Dryopteris* Cutter (1956) has reported a close correlation between the setting off of a large, lenticular apical cell and the determination of the primordium as a leaf. The apical cell in an *Osmunda* primordium is not so large and distinct relative to its surrounding cells as is the case in many other ferns; and the presence of such a cell could not be determined easily under a binocular dissecting microscope. Longitudinal sections of shoot apices show leaf apical cells to advantage; but it is difficult to identify primordia in sequence in such sections. In transverse sections, the sequence is easily determined; but the curvature of the leaf primordia makes recognition of

2. The author is indebted to Dr Carl R. Partanen of the Department of Biological Sciences, University of Pittsburgh, Pittsburgh, Pa, U.S.A., for suggesting the use of these containers on the basis of his unpublished observations on the growth of fern prothallia in sterile culture.

the apical cell in such sections difficult. A combination of these three types of observations, however, has led to the conclusion that the apical cell is always present in the third primordium, but that it can only rarely be seen clearly in the second, although suggestions of its presence are often noted. It is concluded that in the second primordium an apical cell is set off at about the time that the primordium becomes the third, that is, at about the time that a new first primordium is initiated.

The first 10 primordia grown in sterile culture gave results which were strikingly different from those obtained earlier with older leaves. Many of the primordia, particularly at the younger end of the sequence, gave rise to leafy shoots which soon formed roots and ultimately developed into small, complete plants (Figs. 2, 3). Moreover, many of the primordia which developed into leaves also produced roots (Fig. 4) a condition which was rare in older excised leaves. The data given in Table 1 indicate the fates of excised primordia 1 to 10 in one experiment in which all were grown on Knudson's medium with two per cent sucrose. The survival rates of excised primordia in this experiment were typical for a large number of experiments as were the general results obtained. This particular experiment, however, contained an unusually large number of doubtful results, possibly because it was terminated at an early stage (3 months). In the course of these experiments a large number of primordia have been cultured on a variety of media. The results of all of these studies have been grouped in Table 2 without regard for medium, primarily to indicate the extent of the data upon which the considerations to follow have been based.

It is apparent from the data in Tables 1 and 2 that the first 9 primordia are capable, under some circumstances, of developing in a manner which would be expected of a piece of the apical meristem, or of the whole meristem, rather than as would be normal for a leaf. It must be concluded that, in these cases, the primordia had not been irreversibly determined as leaves prior to excision. Although the tendency to produce whole plants (lack of irreversible determination) is clearly greater in the youngest primordia and decreases in progressively older leaves (Tables 1, 2), there is a high degree of variability in the expression of any particular primordium in the sequence. The nature of this variability is such as to render extremely unlikely the interpretation that it is the result of inaccuracy in the recognition of primordia although this might provide a partial explanation. Moreover, in a few cases, a shoot has been produced by a primordium older than some from the same plant which produced only leaves. This variability remains at present unexplained; and it is the subject of investigations now in progress.

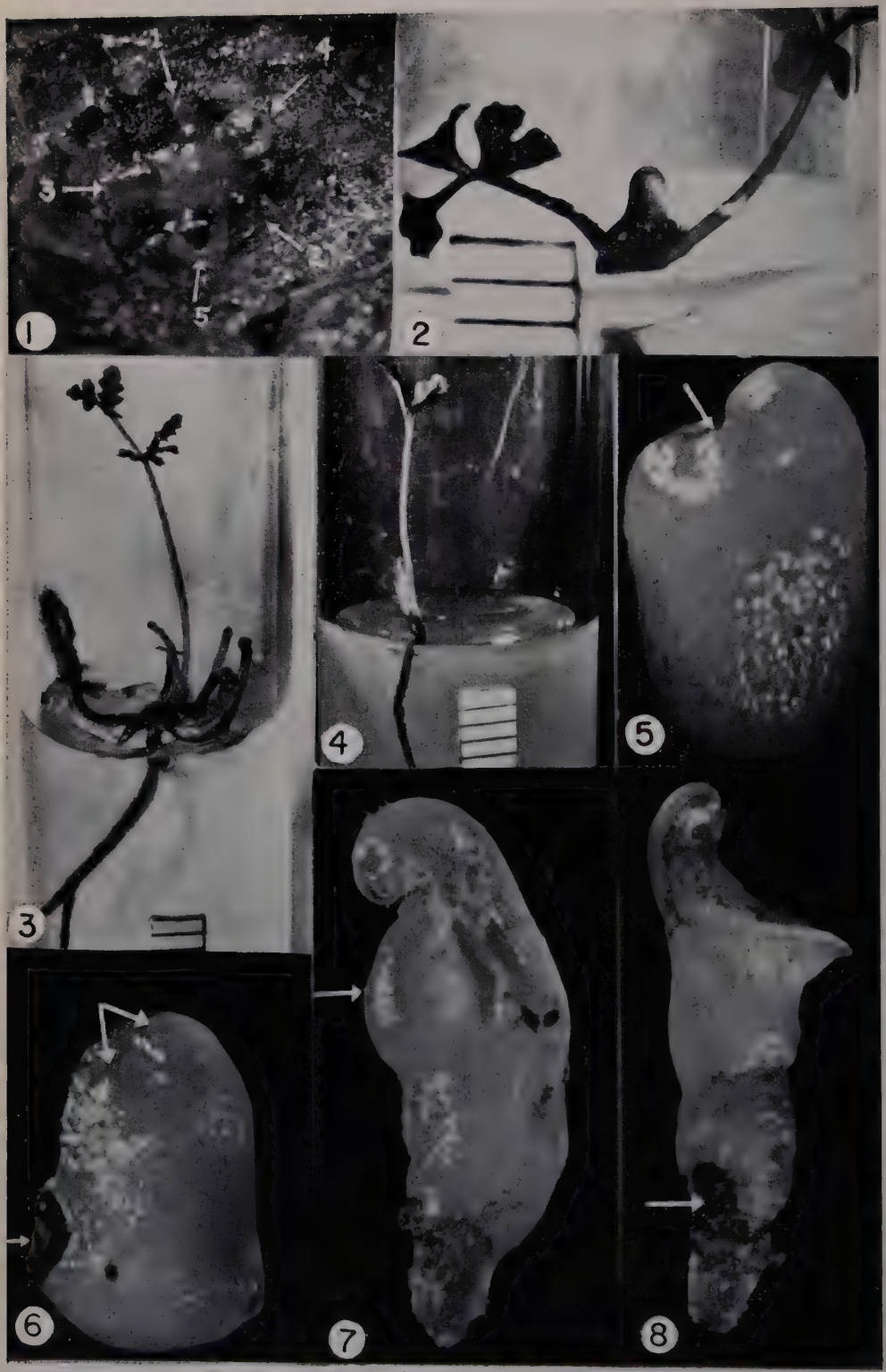
TABLE 1 — FATES OF EXCISED PRIMORDIA IN A SINGLE EXPERIMENT CULTURED FOR THREE MONTHS ON KNUDSON'S MEDIUM WITH TWO PER CENT SUCROSE

Primordium	Shoots	Leaves	Doubtful	No growth
P_1	3	0	2	4
P_2	5	0	2	4
P_3	3	2	5	2
P_4	3	2	3	5
P_5	4	4	2	3
P_6	5	6	1	1
P_7	1	9	1	2
P_8	0	11	1	1
P_9	0	10	0	0
P_{10}	0	8	0	0

TABLE 2 — FATES OF EXCISED PRIMORDIA SUCCESSFULLY CULTURED IN EIGHT EXPERIMENTS. ONLY THOSE CLEARLY RECOGNIZED AS SHOOTS OR LEAVES ARE RECORDED

Primordium	Shoots*	Leaves	Per cent shoots
P_1	8	0	100·0
P_2	18	1	94·7
P_3	19	4	82·6
P_4	30	4	88·2
P_5	28	23	54·9
P_6	14	24	36·8
P_7	10	29	25·6
P_8	7	19	26·9
P_9	2	14	12·5
P_{10}	0	8	0·0

*In many cases whole plants at the time of termination.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THE INFLUENCE OF COMPOSITION OF MEDIUM UPON THE DEVELOPMENTAL POTENTIALITIES OF EXCISED PRIMORDIA — As has already been suggested, excised primordia of *O. cinnamomea* survive and make satisfactory growth on a medium containing only mineral salts and sucrose. The basic medium in most of these experiments has been Knop's solution with 2 per cent sucrose added. Various additions or substitutions have been made in certain experiments, and the results of these treatments may be summarized briefly. Unfortunately, because of the extremely small size of primordia at the time of excision, and even after considerable growth in culture, estimates of growth rate either have been subjective or have been based upon linear measurements only taken at the time of termination. Variations within treatments have made even these estimates difficult to interpret.

In early experiments a mixture of B vitamins was regularly added to the medium. However, it was shown that the presence of these vitamins improved neither the survival of the youngest primordia nor the growth of any primordia to a recognizable extent; and their use was discontinued. Similarly, in certain experiments, coconut milk, both autoclaved and unautoclaved, casein hydrolysate and naphthalene acetic acid were added to the medium with no obvious improvement either in survival or in growth. In fact, there was some indication that the presence of all of these additives in the same medium was unfavorable to the survival of the youngest primordia; but the evidence on this point is not conclusive.

In other experiments variations in the concentration of sucrose in the medium were introduced. Raising the concentration to 6 per cent, the highest level tested, led to increased growth, and reducing the concentration to 0·5 per cent noticeably reduced the growth. Similarly the substitution of Knudson's solution for Knop's enhanced the growth of excised primordia. It seems probably that the value of the Knudson's solution lies in its content of ammonium ions as was the case in earlier experiments on the growth of excised leaves of flowering plants (Steeves *et al.*, 1957); but this has not as yet been investigated. The best growth was obtained on a medium containing Knudson's solution and 6 per cent sucrose.

Perhaps the most striking feature of the experiments in which composition of the medium was altered was that in no case was any consistent change noted in the type of development expressed by the excised primordia. On all media the pattern of development was variable, with an increasing frequency of leaf formation in progressively older primordia. It is for this reason that it has been deemed proper to combine data from all experiments in a single table (Table 2). One of the characteristics of a leaf which appears early in its development is an accelerated growth rate relative to that of the shoot apical meristem. Although the growth of primordia on any given medium was variable, comparison of a very favorable medium (Knudson's plus 6 per cent sucrose) with a very poor medium (Knop's plus 0·5 per cent sucrose) showed striking differences in growth rate on the average, and even more striking differences in individual cases. Yet the establishment of

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FIGS. 1-8 — Fig. 1. Dissected shoot apex of *O. cinnamomea* with the first five leaf primordia (P_1-P_5). × 20. Fig. 2. Complete plant developed from a third primordium (P_3). × 6. Fig. 3. Plant developed from a sixth primordium (P_6). × 2. Fig. 4. Leaf with root produced by a seventh primordium (P_7). × 2.5. Fig. 5. Shoot apex (arrow) with two leaf primordia which has developed directly from a first primordium (P_1). × 60. Fig. 6. Fourth primordium (P_4) showing division of original leaf apex (paired arrows) into shoot apex (left) and leaf primordium. Excision scar (bottom arrow) indicates original adaxial face. × 35. Fig. 7. Shoot development from a third primordium (P_3) showing advanced stage of first leaf and apparent adaxial position of shoot apex with younger leaves (arrow). Root at base. × 25. Fig. 8. Third primordium (P_3) showing development of dorsiventrality after excision. Excision scar (arrow) indicates original adaxial surface. Root at base. × 25.

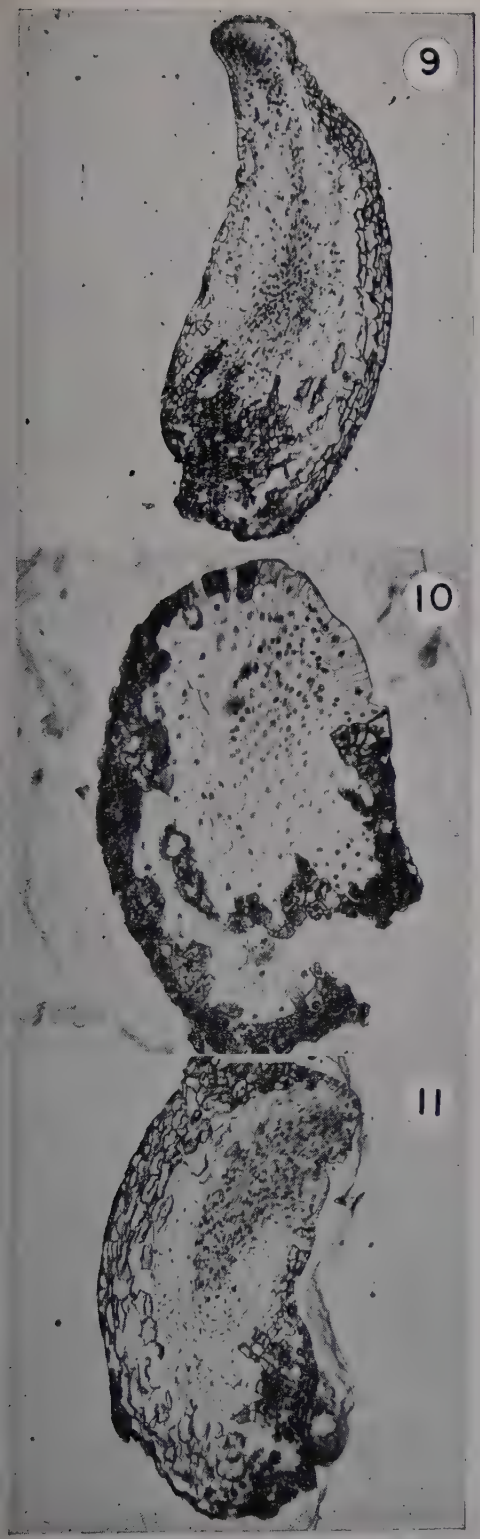
the leaf or shoot pattern of development in excised primordia was independent of these differences.

Patterns of Development in Excised Primordia — It has been pointed out that when the first 10 primordia of *O. cinnamomea* are explanted to sterile nutrient cultures, some develop as leaves while some produce shoots, and ultimately whole plants. It is of interest to know the sequence of developmental events by which these final products come into existence. Of particular importance, in the case of those which produce shoots, is the question of the origin of the shoot apex, whether from the original leaf apex or from another source. By the systematic examination of primordia at successive intervals after explanting it has been possible to obtain information pertaining to this problem. Careful study during the first 2 to 3 weeks in culture has shown that development always takes place from the main body of the primordium rather than from any tissues carried along accidentally in excision or from proliferations stimulated by wounding. It was especially important to know this in the case of the first 2 primordia which were ordinarily excised on small plates of tissue.

When a piece of the apical meristem of *O. cinnamomea* other than a leaf primordium is excised, it ordinarily forms a new shoot apex and quickly begins to produce leaf primordia. The youngest primordium (P_1), which has always in these experiments produced a whole plant, develops in essentially the same manner (Fig. 5). Often the second primordium (P_2) follows the same pattern. Older primordia, on the other hand, require a different interpretation even in cases in which a shoot is ultimately produced. After excision, the primordium apex continues its development for a variable period of time without obvious external change, and without the formation of leaf primordia. As has been pointed out, primordia on the shoot apex have a distinct adaxial curvature; and the leaf apex ordinarily maintains this after excision by continuing without change in the direction of growth. The original orientation of the excised primordium is easily recognized during development by means of the excision scar which ordinarily becomes discolored and sometimes slightly callused. Since the primordium was curved adaxially prior to excision, this scar is a plane surface facing approximately in an adaxial direction. As far as could be determined, the orientation of the primordium on the surface of the medium has no influence upon the direction of growth of the leaf apex.

In some cases the leaf apex continues its development in the manner just described and gives rise to a small dorsiventral leaf (Fig. 9). In such cases, the basal portion of the leaf becomes broad and flattened through marginal growth and the apex subsequently forms a crozier which ultimately uncoils and expands. The frequency of occurrence of this pattern increases in progressively older primordia. In other cases, however, those in which a shoot is produced, the original apex gives rise to two meristematic mounds of approximately equal dimensions (Fig. 6). This does not appear to be a case of the production of a leaf primordium by a shoot apical meristem, but rather the division of a meristem into two equal parts. One of these meristematic mounds, abaxial in position relative to the organization of the original primordium, becomes the apex of a leaf, the first leaf of the shoot. The other mound, adaxial in position, becomes a shoot apex which soon produces a leaf primordium, ordinarily opposite in position to the one just described. Although such small pieces of tissue are extremely difficult to section because of problems of orientation, it has been possible to obtain information in a few cases on the internal organization of primordia in the critical stages just described. An examination of this material has entirely substantiated the interpretation based upon external observation (Figs. 10, 11).

In interpreting the development of a shoot from a leaf primordium in this manner, perhaps the simplest conclusion would be that the original leaf apex becomes a shoot apex and begins to form leaf primordia. There are, however, several reasons for believing that the first leaf is not entirely equivalent to the later primordia in its relation to the new shoot apex. In the first place, as has been



pointed out, its origin is not at all suggestive of ordinary leaf primordium initiation. The first leaf is always in a definite position relative to the organization of the original excised primordium and, in this position, both externally and internally, it continues the plan of organization of the original leaf. This last feature is particularly noticeable in certain cases in which the first leaf is very much advanced in its development over the succeeding primordia. In such cases, the new shoot apex very much resembles an adventitious bud developed upon a leaf (Fig. 7). In fact, this was the interpretation given in the early phases of this investigation. However, the constant position of the shoot apex in all cases and the mode of development render this interpretation improbable.

From these observations it may be concluded that, while very young primordia are not irreversibly determined as leaves, they do, beyond the first or sometimes the second, persist in a leaf-forming pattern of development for a time after excision. In some cases, especially in older primordia, this pattern is followed to completion and a leaf is produced (Fig. 9). In other cases, however, the leaf pattern is not maintained and a shoot apex arises from part of the original leaf apex. In such cases, the original pattern is reflected in the first leaf of the shoot which is not, therefore, equivalent to later leaves formed by the new shoot apex.

It has been pointed out that roots are produced by many of the excised primordia, both those which develop as shoots and those which give rise to leaves. The location and mode of origin of roots do not appear to differ in the two instances. In most cases, a root appears near the base of the developing primordium on the

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FIGS. 9-11 — Fig. 9. Median longitudinal section of a fifth primordium (P_5) developing as a leaf. × 50. Fig. 10. Median longitudinal section of a second primordium (P_2) showing early stage of division of apex into shoot apex (below) and leaf primordium. × 80. Fig. 11. Median longitudinal section of a fourth primordium (P_4) showing later stage of development of shoot apex (below) and first leaf. × 60.

abaxial face. In exceptional cases, however, a root may arise laterally or even on the adaxial face. The roots in all cases arise endogenously close to the developing vascular strand, and emerge to the surface through the surrounding tissues of the primordium. The first indication of root development is the conspicuous enlargement of a nest of cells at the periphery of the vascular strand. At the level of root formation the vascular tissue, in the cases examined, consisted mainly of procambium with a few differentiated tracheids. The nest of cells subsequently gives rise to a root apex, with several apical initials as in the intact plant, which emerges to the surface with evident crushing and displacement of surrounding cells.

The formation of roots by very young excised primordia of *O. cinnamomea* might be interpreted as further evidence of the lack of complete determination. In keeping with this view is the observation that older excised leaves rarely produce roots in sterile culture. On the other hand, the development of adventitious roots closely associated with leaves is widespread among ferns and is found in *O. cinnamomea*. In the intact plant, however, the root arises in the pericycle of the rhizome stele adjacent to the departing leaf trace. The relationship of roots to the excised primordia from which they arise is so different that there can be no question of a root primordium being excised with the leaf.

In a few instances, structures arose from excised primordia which cannot be fitted into the developmental pattern which has been described above. Sussex (1951, 1955) has reported surgical experiments in which, by partial isolation from the apical meristem, an incipient leaf primordium was caused to develop as a determinate but radial organ, a centric leaf. Similar structures have arisen in the present experiments, especially in the younger part of the primordium sequence. Most of the centric structures produced were of limited growth and were pointed at their tips. In one case, however, a second primordium gave rise to a cylindrical axis topped by a circular, peltate lamina. These observations would seem to support Sussex's conclusion that dorsiventrality and determinate growth in leaves are separate phenomena, independently initiated.

In those primordia which gave rise to leaves, it was frequently noted that the extent of dorsiventrality increased during the development of the primordium so that the upper portions were more pronouncedly dorsiventral than was the base. In a few cases, the base, including all of the tissue formed prior to excision, was completely radial in symmetry, externally and internally, with a rather abrupt change to dorsiventrality occurring at some distance above the base. Such observations might be interpreted to indicate that dorsiventrality appeared in the primordium in response to some condition prevailing in the culture system. Without further evidence, however, it would be unwise to draw such a conclusion since the appearance of morphological dorsiventrality might reflect the expression of some physiological gradient imposed upon the primordium before its excision. In a few instances, however, the plane of dorsiventrality was different from that of the original primordium. In one case the adaxial surface of the upper portion of the leaf faced 90° away from the cut base which marked the original adaxial face (Fig. 8), and in another case, 180°. Such scanty evidence as this does not permit of broad generalizations, but it at least suggests that the conditions which bring about dorsiventrality in leaf primordia in this region of the shoot apex might be duplicated in sterile nutrient culture.

Discussion

Sterile culture studies carried out prior to the present investigation (Steeves & Sussex, 1957; Steeves *et al.*, 1957) led to the conclusion that the complex pattern of leaf development is essentially self-controlled within the organ once the primordium has been determined, since an excised leaf can develop in a fundamentally normal fashion in complete isolation from the parent plant. The process of leaf determination sets in motion a chain of morphogenetic events which is self-perpetuating. The new observations reported

here in no way argue against this earlier conclusion; in fact they strengthen and extend it. They show, however, that, at least in *O. cinnamomea*, a leaf primordium is not irreversibly determined from its inception. There is, in fact, a relatively long period of development during which it remains undetermined, or at least incompletely determined, and during which, if it is removed from the influences in the shoot apex which bring about its determination, it may not continue to develop as a leaf. The fact that in some cases it develops as a leaf and in other cases does not, poses special problems which will be discussed below; but it does not alter the basic conclusion. Once, however, irreversible determination has been achieved, the primordium is essentially independent in its subsequent development.

It has been assumed that the excised young primordia of *O. cinnamomea* which produced shoots did so because they had been excised prior to their irreversible determination as leaves. In this view, when the incompletely determined primordium is removed from the influences which normally act upon it in the shoot apex and cause its determination, it fails to develop as a leaf, developing rather in a manner comparable to any undetermined portion of the apical meristem. On the other hand, it could be argued that the developmental patterns studied in these experiments have nothing to do with normal leaf determination, reflecting rather the results of injury incurred during the excision. If the former view is to be adopted, it should perhaps be defended in favor of the latter.

In most cases, following excision, the leaf primordium continues in a developmental pattern not markedly different from that which it had been following before its removal from the shoot apex. Only later does shoot development occur. If injury effects were of importance, it seems unlikely that they would be thus delayed. Moreover primordia older than the ninth have always produced leaves rather than shoots, and their developmental pattern is essentially a normal one. It would be difficult to understand why injury effects are not apparent in these somewhat older primordia, if indeed such factors are of major importance. It is also significant that when a shoot arises from a leaf primordium, it does so in an orderly fashion and from the apex of the leaf itself. There is no evidence of a breakdown of organization such as might be expected in a simple wound response. In certain cases accidental injuries of considerable extent do occur and the original leaf apex may be destroyed or seriously damaged. When this happens, buds often do arise from other parts of the primordium; and in some cases several such buds may be formed from a single explant. Such developments as these would appear to be the true injury responses; and they are quite different from the ordinary course of events in excised primordia.

Perhaps the most convincing evidence for considering that the results presented in this report do pertain to normal leaf determination is their close comparability to the results of other workers obtained by surgical methods, although these results too might be suspect of including injury effects. The observations of Sussex (1951, 1955), Wardlaw (1949b, c) and Cutter (1954, 1956) all indicate the existence of a period in the early development of a leaf primordium during which its normal characteristics have not become completely fixed and may be modified by experimental manipulation. Sussex (1955) was able to show that the leaf characteristics do not all become fixed at the same time; and the occurrence of a certain number of radial but determinate organs in the present experiments provides a close parallel to his work. The major difference between the results of the sterile culture studies and those of the earlier surgical experiments is the duration of the period during which the fate of a primordium remains incompletely determined. In Cutter's experiments with *Dryopteris* (Cutter, 1954, 1956), which of all the surgical studies most closely parallel the present work, the third primordium was the oldest which could be caused to develop as a shoot; and the setting off of an apical cell appeared to correspond to the establishment of a fixed pattern in the primordium. In sterile culture studies with *Osmunda*,

primordia as old as the ninth have produced shoots and the setting off of an apical cell bore no obvious relationship to the irreversible determination of the primordium. The information available does not permit a resolution of these differences at the present time. It may be suggested, however, that differences between species could be of importance, and in this connection it should be recalled that in culture studies on leaves of flowering plants (Steeves *et al.*, 1957) the second primordium, the youngest which survived, always produced a leaf. On the other hand, it may well be that in the surgical studies not all of the formative influences which operate upon a developing primordium in the region of the shoot apex have been eliminated, whereas in sterile culture, the primordium, completely isolated from the parent plant, is completely removed from all formative stimuli. In any event, it would seem to be safe to proceed with the assumption that the phenomena observed in these investigations do pertain to the problem of normal leaf determination.

One of the most striking features of the development of excised primordia is the variability in their growth patterns. In the case of any given excised primordium it is possible, on the basis of past experience, to state the probability that it will develop as a shoot or as a leaf, but, from the second to the ninth, any individual could become either. At present no explanation for this variability can be put forward; and it remains one of the major problems for future investigation. It has already been pointed out that inconsistencies in the recognition of primordia could not explain the variation; and, since experiments have been carried out at various times of the year and considerable differences can be found within a single experiment, it is also certain that seasonal differences are not involved. The variability might be explained, in part at least, by differences between individual plants in whatever the factors are which control leaf determination. A careful study of the first 10 primordia in a large number of plants might well reveal structural differences which could be correlated with the differences in development after excision. It has been shown that the setting off of a distinct apical cell does not correspond to irreversible determination; but it is possible that some other aspect of differentiation, such as the attainment of a certain stage of development by the vascular tissue, could have an important determining role. There is no reason to suppose, however, that differences in the factors which control leaf determination must necessarily have structural manifestations.

On the other hand, the possibility must be considered that the variable development of excised primordia results from variations in handling during and after excision which are not as yet understood, or even recognized. Since major differences in the composition of the culture medium, which resulted in some cases in large differences in growth rate, did not appear to have any effect upon the fate of excised primordia, it is difficult to envision the sort of factors which might be important. Clearly no conclusion in this matter can be reached until an exhaustive study of the influence of a variety of chemical and physical factors upon the fate of excised primordia has been carried out. Leaf determination can, of course, be much more readily studied by the sterile culture method if chemical or physical factors can be found which do influence the fate of excised primordia.

A study of the development of excised leaf primordia has shown a marked retention of leaf-like characteristics particularly in the early stages of growth after excision, even in those primordia which ultimately develop as shoots. It is impossible to avoid a comparision with the development of the embryo of many ferns including that of *Osmunda*. The independent origin of the first leaf in the embryo and its predominance over the shoot apex in early development (Wardlaw, 1955b) suggest a similarity to the development of an excised primordium. On this basis it might be suggested that the incompletely determined primordium develops in isolation as an embryo might; and the implications of such a suggestion might be far reaching indeed. Such a conclusion, however, is eliminated by a closer examination of the facts. The predominance of the leaf in

early development is much more characteristic of older primordia than it is of younger ones, and an excised portion of the apical meristem does not show it at all. Since a piece of the apical meristem or a very young leaf primordium should certainly be more like an embryo than should an older primordium, the comparability observed in this case would seem to be merely coincidental.

Although the information at hand does not permit of any far-reaching conclusions, it is possible to formulate a working hypothesis which is in accord with observed fact and which can point the way to future investigations. In the process of leaf determination, a specific morphogenetic pattern is established within a restricted portion of the meristematic tissue of the shoot apex, the leaf primordium. In some way the primordium acquires the " information " which causes it to develop as a leaf. A piece of the apical meristem or a very young primordium, when isolated, gives rise directly to a shoot apex. The formation of a shoot apex would thus appear to be the most direct expression for a piece of meristem which has received no, or very little, leaf-forming " information ". A leaf primordium excised at a later stage has presumably received more of the " information " since it has been exposed for a longer time to the formative influences operative in the shoot apex; and it continues to develop as a leaf. If it contains enough " information " to enable it to continue to the point of irreversible determination, the point at which it becomes independent of external leaf-forming stimuli, it will give rise to a leaf. If it does not, it will sooner or later revert to the more direct expression and give rise to a shoot apex. Its earlier leaf-development phase will, as has been seen, be evident in the first leaf of the shoot. The leaf-forming " information " must be a positive acquisition by the primordium, and not simply a lack of inhibition.

Although there is no direct evidence to indicate it, the leaf-forming " information " seems likely to be of a chemical nature, a single substance or possibly a combination of substances. The existence of leaf-forming substances has been considered earlier in the interpretation of other observations (Wardlaw, 1949a). In chemical terms, the fate of an excised primordium would depend upon its content of a leaf-forming substance. Such a substance would, until exhausted, cause the primordium to develop as a leaf, after which time the primordium would revert to the simpler expression and give rise to a shoot apex. On the other hand, if a sufficient supply of the substance were present to carry the primordium to the point of irreversible determination, possibly the point at which it acquired the ability to synthesize its own leaf substance, it would produce a leaf rather than a shoot. The seeming appearance of dorsiventrality in an excised primordium, which has been described earlier in this report, might at first appear to be in conflict with this interpretation. If, however, it appears after irreversible determination has occurred, it might be regarded as one of the manifestations of a plentiful supply of leaf-forming substance. The variable expression of excised primordia is likewise not in conflict with this hypothesis. Presumably individual plants could vary in the concentration and distribution of such a substance, and certainly differences in handling of primordia during and after excision could influence its synthesis or its destruction.

The interpretation of these experimental results in terms of a chemical substance constitutes at best a working hypothesis, and much further work will be required to prove or disprove it. An extensive search must be made for chemical and physical conditions which will influence the development of excised primordia. Such a study should certainly include an investigation of substances which have been reported to have an influence upon leaf development *in vivo*. It should also include a study of the influence of excised primordia upon each other, or of the influence of an excised shoot apex upon excised primordia in combined cultures. It is not to be hoped that an understanding of leaf determination will be achieved easily, or that the mechanism is a simple one. It does appear, however, that the method of sterile nutrient culture is an extremely favorable technique for an attack upon this problem.

Summary

The first 10 visible leaf primordia of the fern *Osmunda cinnamomea* have been excised and cultured on a nutrient medium of simple composition. In contrast to results previously reported with older leaves, many of these primordia gave rise to leafy shoots and ultimately to whole plants. The formation of shoots was most frequent at the younger end of the sequence and decreased in progressively older primordia; but there was variability in the expression of any primordium between the first, which always produced a shoot, and the tenth, which always gave rise to a leaf. Variations in the culture medium which included changes in sucrose concentration, alteration of the mineral salt solution and the addition of complex substances did not appear to influence the shoot or leaf expression of excised primordia. Detailed examination of cultured primordia at various stages after excision has shown that, when a shoot is formed, its apex arises from the original leaf apex. The first leaf of the shoot, however, reflects the organization of the orginal primordium and seems not to be entirely equivalent to later formed leaves. These observations make it clear that primordia in *O. cinnamomea* can be excised and cultured at a stage which precedes their irreversible determination as leaves. The results are discussed in terms of the possible role of a leaf-forming substance in the process of leaf determination.

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ON *CARRPOS* I

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Some five years ago Professor D. J. Carr, now of Belfast, Northern Ireland, described a new liverwort, assigned to the Marchantiales, from a saltpan in the interior of Victoria, Australia. The original name, *Monocarpus sphaerocarpus* Carr (1956), has been changed by me to *Carrpos sphaerocarpos*; the various technical reasons for this have been set forth in a separate note (Proskauer, 1961b). When I saw Dr Carr's paper, I became greatly interested in the organism and started to correspond with him. In the summer of 1959 he kindly brought me a dried field sample from Australia. From this, and some spores of the type material sent later, it has been possible to establish cultures here. The present paper deals mainly with the development and vegetative structure of the gametophyte. The thallus proved to be rather more complex than described, a discrepancy which I explain by the type field material having been both somewhat depauperate, and precociously fertile, with the expanding archegoniophore compressing the vegetative tissues. I have been able to confirm the bulk of the other factual statements in the original description. However, the sporelings figured there would clearly seem to have resulted from leptosporangiate fern spores, which only too easily find their way into cultures. (N.B. Counter to Carr's statement, *Sphaerocarpos* does not produce stalked glands of the type shown.) Further, I strongly doubt that meiosis in a sporophyte is other than simultaneous, but so far lack suitable material to test this. My interpretations of the facts, on the other hand, differ largely from those of Carr. I do not see *Carrpos* as phylogenetically intermediate between Sphaerocarpales and Marchantiales.

The only starting materials available were raw field soil samples, containing dried spore bearing plants of *Carrpos*, from the following two localities:

(1) "Bare mud of a saltpan, by the side of the Calder Highway at Yatpool, adjacent to Red Cliffs", northwestern Victoria, Australia. Leg. Mrs S. G. M. Carr, viii. 1955. Type locality (cf. Carr, 1956). A small sample, labelled "Isotype material" was received from Prof. Carr in January 1961. Soil particles, very fine, pale reddish brown in colour. The scant material of *Carrpos* present in it consisted of very small plants bearing sporophytes with mostly immature spores.

(2) Also from near Red Cliffs, but a spot different from the type locality.

Received from Professor Carr, viii. 1959. Soil brick red, with fine sand particles. Thalli and sporophytes larger than in (1). Spores mostly mature. Thalli dried white, with white salt incrustation.

The plant has been found in Victoria in the additional localities of Nowingi (Carr, 1956), and at the Raak, Mildura. But it occurs also much farther afield, for Mrs Carr found some at the margins of saltpans in Western Australia (Carr, in litt.).

Culture Methods

During a four month period, beginning September 1959, a great variety of unsuccessful attempts were made by myself and various students to induce spores from locality 2 (*see* above) to germinate. The media used included the original soil from the locality wetted with distilled water; regular loams; soil from a salt marsh on the Marin County coast of San Francisco Bay; a stale soil sample from the Cape Flats, South Africa; our standard nutrient agar (*see a* below); that agar with a thin film of supernatant seawater; etc. Further, certain samples were subjected to refrigeration or freezing for various periods. I concluded that there was some kind of germination barrier, and evolved a technique to crack and remove the outer colored spore coats by rolling the spores. The protoplasts, within the remaining smooth translucent coats, were full of reserve oil and seemed healthy enough. This material was now sown on standard agar. On this a few of the treated spores developed very short germ tubes but died before undergoing divisions, or after the first few divisions. I concluded that not only did the outer spore coat present a barrier to germination, but that some unusual external factor also had to be provided, as the germlings seemed to die as soon as they were presumably " running out " of their own internal environment. Although the original, but possibly strongly leached, soil had a slightly acidic reaction on wetting, one possibility was to try alkaline media. The first positive result, with the indication that spores can be made to germinate submersed in water, came from an old culture submitted to a variety of treatments (*see b* below). At the end of December 1959 a very small number of healthy germlings began to appear in it.

A list of the media used with varying degrees of success follows. All were used in 9 cm diameter, 5 cm high " Pyrex " glass crystallizing dishes with Petri dish lids. These dishes were filled to about half height with the solid medium. They were kept on the sill of a northwest window, under a temperature range of 17-22°C. Earlier indicator paper *p*H measurements having proved faulty, the only measurements listed are those made by electric meter.

(*a*) Our standard agar. Per liter of distilled water: NH_4NO_3, 0·2 g; $CaCl_2$, 0·1 g; KH_2PO_4, 0·1 g; $MgSO_4.7H_2O$, 0·1 g; $FeCl_3.6H_2O$, 0·05 g; agar, 10 g. Since the beginning of 1960 we have been replacing the ferric chloride by 1 ml of the " Hutner et al." fully chelated trace element solution (Stein, 1958). This medium, including the chelated trace elements but without the agar, has a *p*H of 6·0.

(*b*) Agar *a* covered at sowing (9 Oct. 59) with thin film of seawater, washed with water after absorption of salt and covered 1·5 cm deep with distilled water (11 Nov. 59); replaced water (7 Dec. 59) by 1·5 cm depth of modified " De " *Nostoc* medium: K_2HPO_4, 0·2 g; $MgSO_4.7H_2O$, 0·2 g; $CaCl_2$, 0·1 g; chelated trace elements; distilled water 1 l.

(*c*) Agar *a* covered 1·5 cm deep by a " Benecke " salt solution as in *f*.

(*d*) Agar *a* covered 1·5 cm deep by a " Benecke " salt solution as in *f*, but with " Hutner et al." (*see a*) micronutrients instead of citrate.

(*e*) Agar *a* covered 1·5 cm deep by an aqueous extract (*p*H 7·4) of an artificial soil mix, U.C. mix CI(C) (Matkin & Chandler, 1957, p. 73), in which both the regular lime components had been doubled in quantity.

(*f*) Bauer's modified Benecke agar. Per liter of distilled water: NH_4NO_3, 0·1 g; $CaCl_2$, 0·01 g; K_2HPO_4, 0·0175 g; $MgSO_4.7H_2O$, 0·01 g; ferric citrate, 0·003 g; agar 10 g.

(*g*) Agar *f* covered 0·5 cm deep by distilled water.

(*h*) Agar *f* covered 1·5 cm deep by *Nostoc* medium (*see b*).

(*i*) Agar *f* covered 1·5 cm deep by a " Benecke " salt solution as in *f*.

(*j*) Agar *f* covered 1·5 cm deep by " Benecke-Hutner " solution as in *d*.

(*k*) Our standard artificial soil mix, U.C. mix CI(C) or CII(C) (Matkin & Chandler, 1957). Watered, when necessary, alternately with distilled water and a salt solution as in *a*, with " Hutner " micronutrients.

(*l*) A soil sample from: High beach line near Rush Creek, south side of Mono Lake, Mono County, California, *p*H 8·8. Kindly collected by Mr D. Mason. Mono Lake is undrained, and its shores include alkali desert and volcanic habitats. Watered with distilled water.

(*m*) A soil mixture of $\frac{3}{4}$ *k*, $\frac{1}{4}$ *l*.

(*n*) An even soil mixture of *k* and *l*.

(*o*) A soil sample from: Danburg beach, below Maloney's cabin, Mono Lake. *p*H 8·5. Leg. Mr D. Mason.

(*p*) Vermiculite, watered as under *k*.

Plants brought past the germling stage (*see* below) have been satisfactorily carried in stock culture on media *a* and *p*.

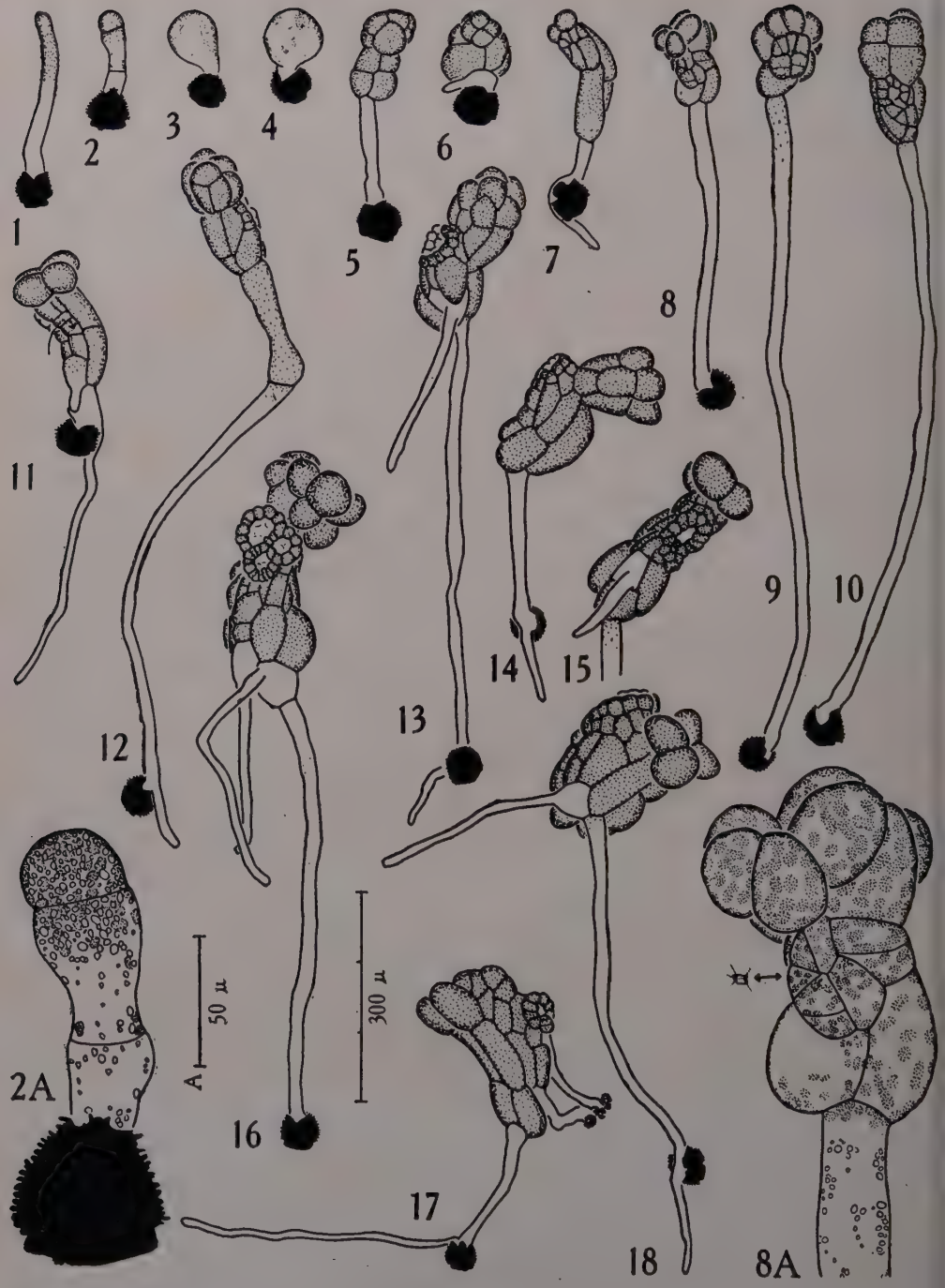
Observations

The material is difficult to handle. Not only are the cells delicate and readily damaged, but even the larger thalli (which in reality are still minute) have most awkward shapes. The microtome proved inferior to needles and hand razor. Every one of the illustrations was prepared from fresh material. For this the thalli often had to be propped up and carefully irrigated with water. As even the best special photographic lenses at the required magnifications lack the requisite depth of focus, illustrations made with a new type Carl Zeiss drawing apparatus were used throughout. Unless otherwise stated, all observations and illustrations were made from cultures started with spores from locality 2. The italic letters in text and legends refer to the culture media listed in the previous section.

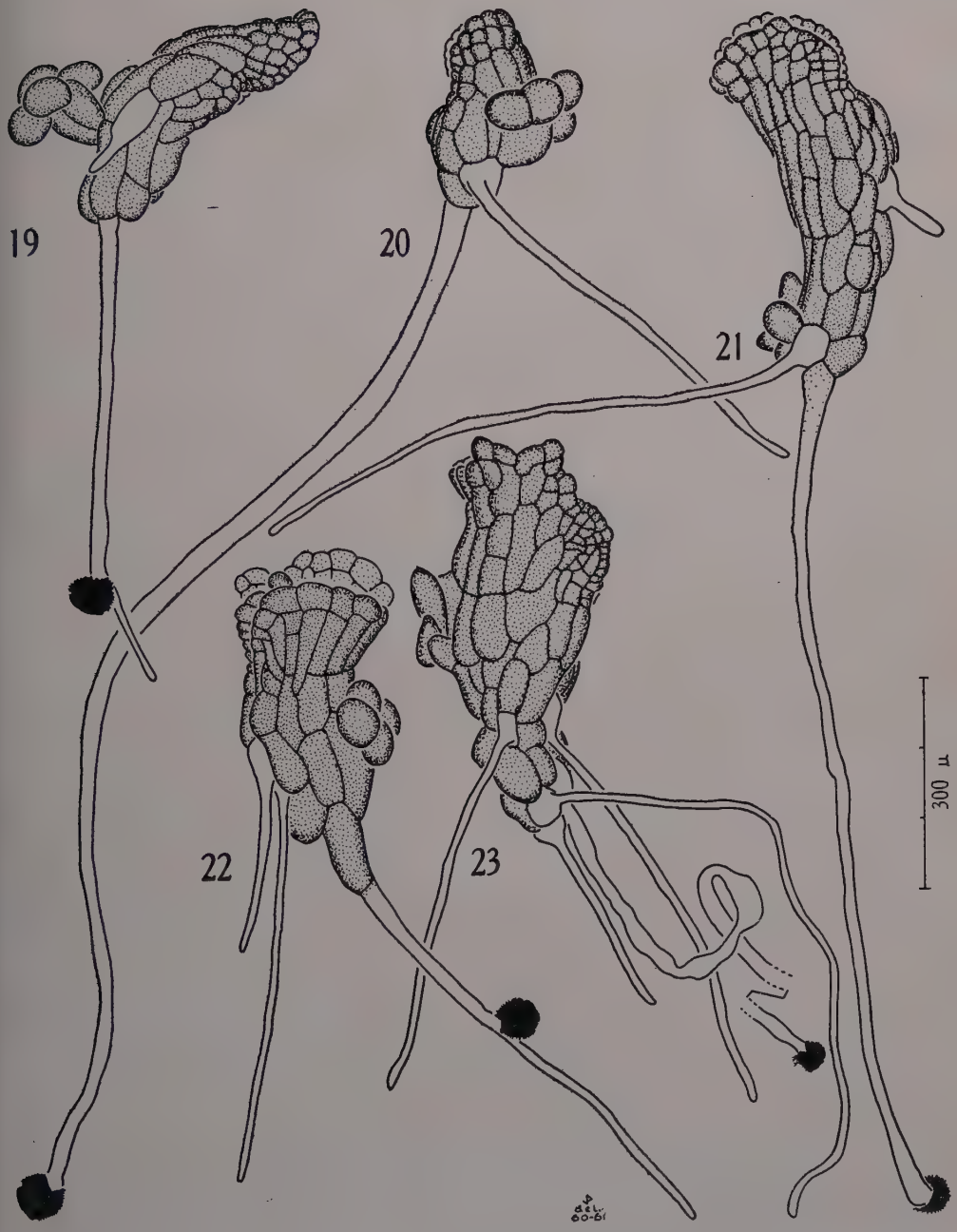
Germination and Structure of Thallus — Mature spores range from a deep to a blackish wine red in colour. Germination is invariably from the centre of the outer face. The spores in Figs. 1-23 have been drawn in silhouette to emphasize this. Their side view (cf. Fig. 2A) is very characteristic. That the smoother face is indeed the inner one has been verified on spore tetrads which had not yet broken up.

Germination has been observed only in the supernatant liquid media *b*, *c*, *d*, *e*, *g*, *h*, *i* (but, for unknown reasons, not in the single dish of *j* tried) and the solid media *l*, *m*, *n* and *o*. With the very limited amount of spore material available it was not possible to run rigorous tests. Indeed, I was glad to get any germlings. In general, spores from different capsules of the field material were mixed for sowings, to compensate for possible differences in degree of maturity. Among the solid media, *l* proved good, both for germination and development, but the other three produced but an occasional germling. The liquid media *d*, *e*, *g*, *h* and *i* permitted germination rates of over 50 per cent (considerably better than that even of soil *l*), and all yielded some healthy plants. In *g* and *i* there was an especially wide range of germling variability, with the subsequent death of the more abnormal germlings. The media *d*, *e* and *h* gave the best ultimate results.

A germ tube grows out through an irregular break in the outer layers of the spore wall (Fig. 1). The length of the germ tube varies greatly, even in individual cultures; occasionally the tube is almost suppressed (Figs. 1-23). The submersed cultures that produced the best ultimate yields of normal healthy plants (*d*, *e*, *h*) had a high proportion of very long germ tubes. On the solid media germ tube length (Figs. 7, 11, 17) seemed to depend on how much an individual spore (none were buried) was shaded by adjacent large soil particles. Figures 1-6 were drawn on the same day from germlings from submersed culture *g*. This culture produced some healthy plants in the long run, but most of those germlings that started as blisters (Figs. 3, 4) died within a fortnight without dividing and there were many abnormal later stages. (Abnormality expressed itself by failure to produce compact organized tissue,



FIGS. 1-23 — A series of stages from germination of the spore to the expansion of the first actual air chamber. In Figs. 2A, 8A, both oil droplets and plastids are drawn. The sketch appended to Fig. 8A shows a schizogenous cavity opening by a minute pore. Figs. 3, 4, 6. Abnormal



germlings. Most blister germlings like those in Figs. 3, 4, failed to divide and died. Fig. 19. The unusual direction of curvature is believed due to the germling floating freely in the liquid medium. (Figs. 17, 23. Loc. 1, isotype; the rest loc. 2. Figs. 1-6: medium *g*; Figs. 7, 11, 17. *o*, Figs. 8, 9, 14, 19, 20, 22, 23. *e*; Figs. 10, 12, 13, 15, 18. *h*; Figs. 21. *b*.)

formation of secondary germ tubes from some of the cells, etc.)

The germ tube cytoplasm carries with it many small oil globules from the spore; only in the tip region are green plastids found (Figs. 1, 2A, 8A). Division of the tip of the germ tube normally leads to the formation of an erect column of cells, composed of distinct upper and lower parts (Fig. 5). The upper part most commonly is composed of two tiers of cells (Figs. 10, 16), but frequently there is only a single tier of four or two cells (Figs. 7, 11, 15). A series of divisions, variable in sequence, now occurs on one side of the lower part of the column, near its upper end (Figs. 7-9). Near the center of this patch of cells, at the point where four or more cells meet, a schizogenous hole is formed. It is not clear (as usual in these cases) whether the split originates at the surface, proceeding inwards, or whether it first appears internally. The sketch appended to Fig. 8A, drawn in good faith, was made when I examined the living specimen with an oil immersion objective after drawing and before lightly staining it in methylene blue to confirm the details. It shows in solid outline a minute internal space, opening by a minuscule pore to the outside. Superimposed in dotted lines is the surface cell wall pattern for orientation.

The pore enlarges by expansion of the neighbouring tissue and division of the cells bordering it (Fig. 10). At this stage the column is still in its original axial alinement (Figs. 11, 12). But the tissue around the pore continues to divide and begins to grow more or less vertically upwards (Figs. 13, 14, 18, 20) until a tall structure is produced (Figs. 21-23), composed largely of the funnel-shaped walls of this first air chamber. During this expansion of the first chamber the symmetry of the germling becomes upset. The cells of the original upper part of the germling column are pushed aside (Figs. 14, 18) and are finally relegated to a lateral position near the base, forming a rather ridiculous little " tail ". The commonest and most characteristic appearance of the tail is that shown in Fig. 19. (This germling shows an abnormal direction of curvature of the first chamber relative to the tail. It is believed to have floated freely in the culture liquid, presumably with the funnel upwards; note the direction of the developing rhizoid near the tail.) Tails developed from single tiers of cells usually appear as in Fig. 22; sometimes their cell arrangement is somewhat irregular (Fig. 23, locality 1, isotype, but similar ones were found in locality 2 cultures); the beady half cingulum in Fig. 20 is rather unusual.

I wish to draw attention to the fact that it is characteristic for the terminal cells of the tail to be broadly spread out from one another (Figs. 8A, 18, 19, 22).

Rhizoid formation may be delayed until this stage. Usually, but by no means always, a rhizoid is formed from the base of the germ tube, without being cut off by a wall. Further rhizoids are derived from rhizoid initials, which become recognizable as such by their pale color.

The second air chamber is initiated in exactly the same manner as the first. A hole is formed where four or more cells abut near the *lower* (in terms of the original orientation of the germling column) margin of the first chamber, and separated from this usually by but a single cell. Figure 15 illustrates this clearly in a somewhat precocious case, usually the initiation of the second chamber occurs slightly later, during the expansion of the first. Further chambers similarly are initiated still farther down (Fig. 16, exceedingly precocious specimen). Although some subsequent chambers have been initiated, the deep funnel of the first chamber constitutes the major part of the germlings in Figs. 19-23. They all are drawn in side view, but the odd cell, shown darker, near the left side of the top of the funnel in Fig. 22 is in fact part of the septum between the first and second chambers, and allows an estimate of the dimensions.

Surprising though it may seem, the details of normal development outlined above are identical in plants grown submersed in liquid culture and subaerially. The liquid cultures were not successful much beyond this point, and growth of material from them was continued by decanting or evaporating the supernatant liquid, or transferring the plants directly to soil or regular agar medium. Media such as *a*, and *k*, which will not allow

germination, are now acceptable to the plants for further growth.

The first chamber had upset the symmetry of the germling by its expansion. The second chamber in turn overtops the first, and its expansion and that of the underlying solid tissue causes the first chamber to curve more in the direction of the tail. As more chambers are formed the process is continued and the plant grows more or less steeply upwards, with the air chambers arranged in an arc as seen in side view (Figs. 24a, 25a). The first one or two chambers occupy the full width of the thallus, then we find chambers in two ranks, then smaller chambers in greater number (Figs. 24a, side, and b, top view of same plant). The plant shown happens to be fertile, but sterile plants even larger than the fertile one in Fig. 26 occur, especially on media *a* and *p*. Such larger plants may be repeatedly dichotomized and the lobes may be broader and thus occupied by more chambers. All the chambers are wide open; in no case is there any roofing. The chambers nearer the apex are sloped obliquely backwards into the thallus (Figs. 24a, 25a, 28, 29). The chambers lack photosynthetic filaments, but lamellae and incomplete or complete septa often occur, presumably all as the result of secondary chamber formation.

The outer walls of the marginal air chambers of the thallus tend to grow to a slightly greater height than the other walls. When sexual maturity is reached, and it is not clear whether there is any direct relationship between the two phenomena, this growth of the outer walls is greatly exaggerated, and they begin to curve inwards (Figs. 24b, 25b, 26), giving the mature plant its characteristic " rolled up " appearance. Indeed, sometimes the margins meet and overlap. As the free outer faces of the walls in question represent morphological " lower " surface tissue, almost all the exposed surface of the thallus now is lower tissue, albeit green and photosynthetic, and the air chambers open into the secondarily delimited cavity. In fact, the situation is essentially as in those Marchantiales which show drying curvatures, when these are in the rolled up condition. Only here the state is permanent, not reversible.

Many attempts were made to gain information on the actual apical organization. All of them showed that chamber expansion occurred in close proximity of the growing point, with the entire upper tissue being converted into chambers separated by largely unistratose septa. Finally, I obtained a surface section (Fig. 37) which seemed almost too good to be true. It came from the still sterile apex of an isotype plant, in general appearance rather like, if somewhat larger, than the plant in Fig. 24a. The drawing was made from the living tissue, with later confirmation of detail by oil immersion lens and light staining in methylene blue. At the extreme top of the figure are cells belonging to the morphologically lower surface of the thallus. Then comes a row of cells, constituting the forward edge of the flat upper surface plateau. This series of cells is in the form referred to in the olden days as a row of apical initials, or the rather more descriptive German name of " Scheitelkante ", but closer inspection suggests that in fact it represents a system of conjugate apical cells as described by Burgeff (1943) for *Marchantia*[1]. This system may possibly be established quite early; it is possible to interpret most of Figs. 7-10, 15-17, thus, but I am too strongly aware of the many possible pitfalls and fallacies in the game of designating cells as " apical " to commit myself. Figure 37 shows pore initiation by a split at the point of juncture of four superficial cells just behind the apex. The proximity of larger chambers suggests that the cells bordering the pore divide rapidly.

There is no trace of mucilage hairs near the apex or anywhere else, nor are there scales on the lower surface of the thallus.

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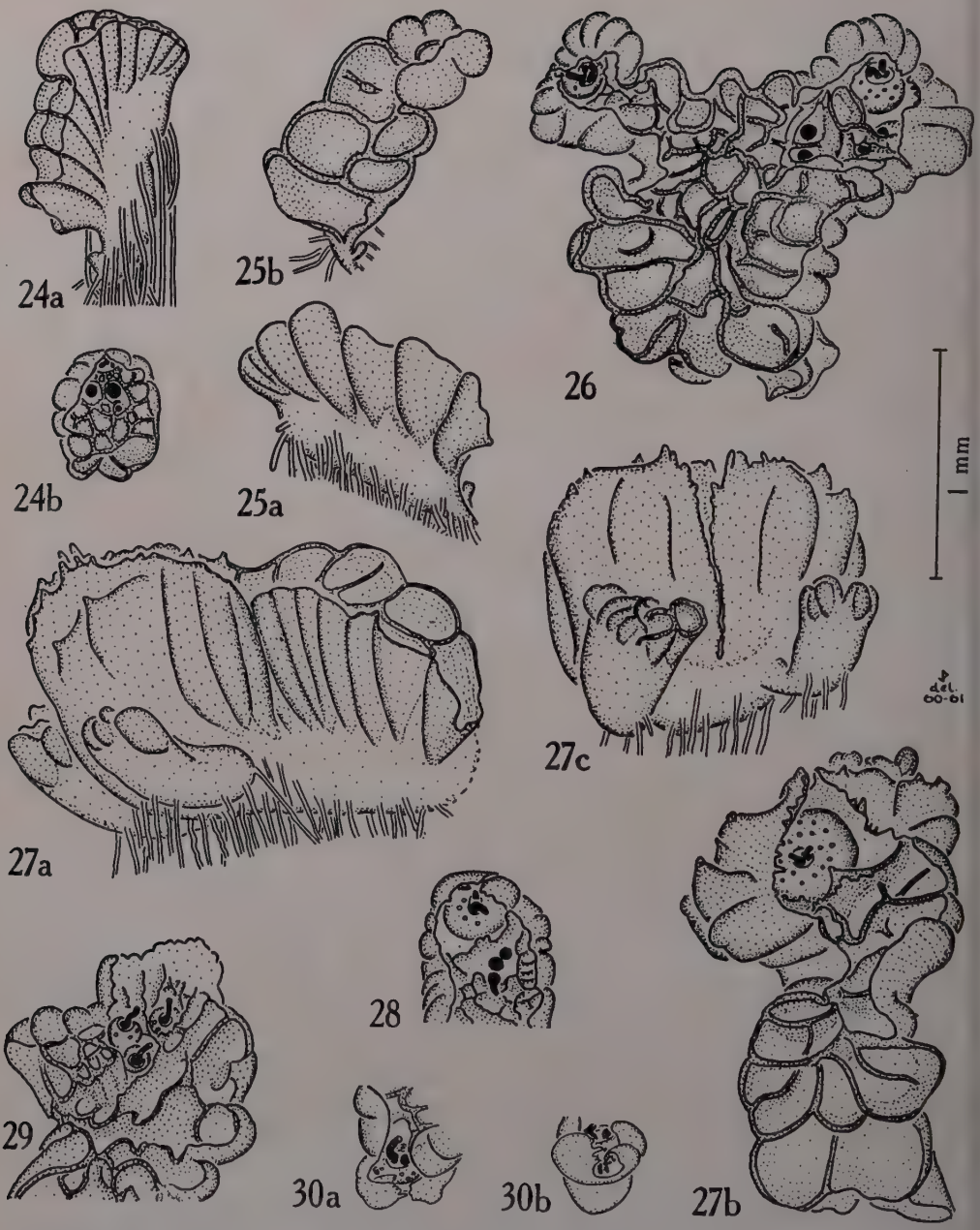
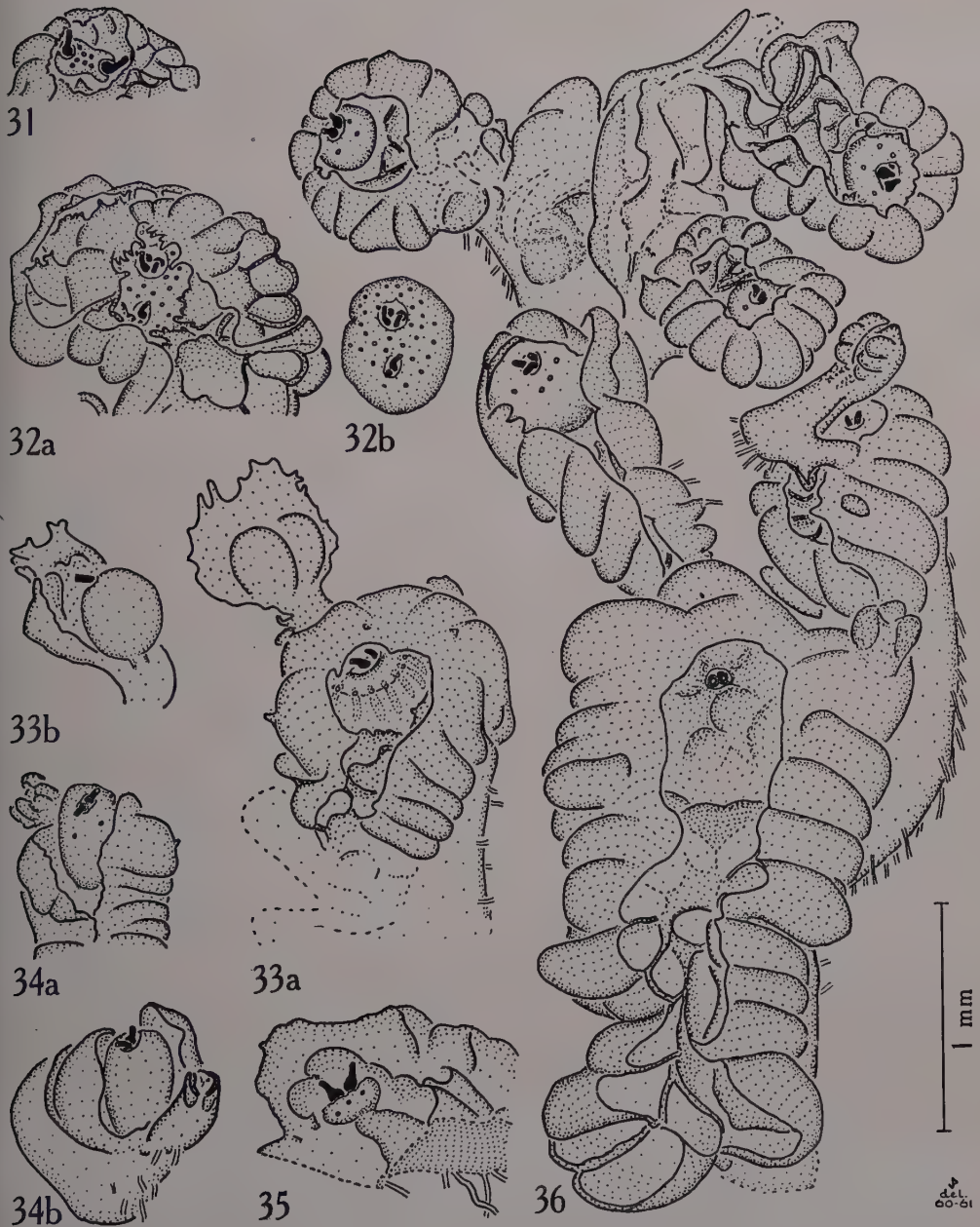
1. The concept of conjugate apical cells at first sight is rather preposterous, and I had strong doubts when first I read about it. However, these doubts have disappeared since I realized that such conjugate apical cells are characteristic of all the Anthocerotales too. In the genera *Dendroceros* and *Megaceros* of that group they can indeed be studied generally without any special manipulation. Conjugate apical cells, but of quite a different sort, also occur at one stage in the sporeling of *Riella* (cf. Proskauer, 1955, Fig. 30).



FIGS. 24-30 — Fig. 24a, b. Side and top views of sporeling with antheridia and developing archegonia near apex (*l*). Fig. 25a, b. Side and top views of sporeling. Two developing archegonia were found inside tip (*k*). Fig. 26. Medium-sized dichotomized plant, with typical fertile tips with antheridia and archegoniophores (*m*). Fig. 27. Plant with two ventral sprouts. a. Side view. b. Top view. Archegoniophore. c. Front view. Note deep split in front of pouch, teeth on pouch margins. The left ventral sprout contained male and female organs (*m*). Fig. 28. Tip of plant with archegoniophore, two antheridia, and a " misplaced " archegonium. (From a patch kept floating on water for a fortnight.) Fig. 29. Tip of plant. The hindmost, non-terminal archegoniophore lacks carpocephalar air chambers (*l*). Fig. 30a, b. Top and front views of one tip of a big branched plant, showing scale-like developing pouch valves (*k*). (All Figs. loc. 2.)



FIGS. 31-36 — Fig. 31. Tip of plant, with a young two-sectored carpocephalum. (From a patch kept floating on water.) Fig. 32. Tip (a) of plant, with an abnormal double pouch, and a two-sectored carpocephalum (b). All the archegonia were old and aborted (*l*). Fig. 33a. Main thallus with pouch closed in front, carpocephalum with pores opening in a ring. The ventral sprout, shown from below in Fig. 33b, has an incomplete pouch and no air chambers in the carpocephalum (*l*). Fig. 34. Top (a) and front (b) views of tip of thallus with archegoniophore and ventral sprout (*k*). Fig. 35. Tip of thallus with most of one side removed, showing developing archegoniophore with massive stalk, and inside of pouch (*k*). Fig. 36. Thallus with a series of ventral sprouts. The top of the carpocephalum in the primary segment had proliferated (*k*). (All Figs. loc. 2.)

The older protoplasts may contain small oil globules, stainable with Sudan III dye, which are not associated into "oil-bodies". All the rhizoids are anchoring rhizoids. They are unicellular, have smooth walls, and are fairly straight to somewhat wavy (Fig. 27a).

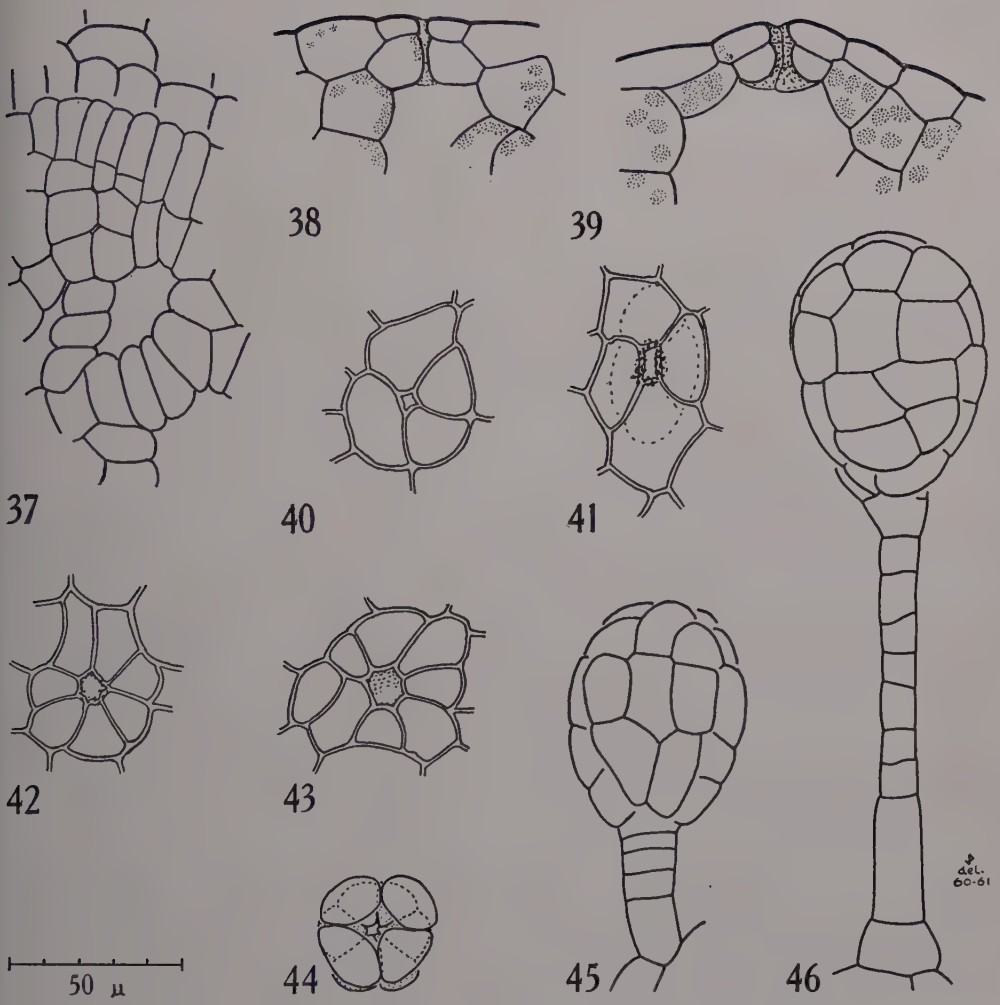
Sex Organs — *Carrpos* is monoecious. Although sex organ development occurs even on agar (*a*), it was most prolific on the soils *k*, *l* and *m*, and on these it commenced when plants were still minute (Fig. 24b).

The antheridia have ovate bodies and a uniseriate stalk of variable, though usually disproportionally great, length (Figs. 45, 46). The jacket cells lose their green color at maturity and become whitish. The stalk cells turn brown and appear to necrose prior to maturity of the antheridium, in the manner of the sporophyte stalks of *Riella*, *Sphaerocarpos*, and, according to Carr (1956), those of the present plant, as well as its archegoniophore stalks. Immature spermatids have been obtained from antheridia, and empty antheridia have been seen, but antheridial discharge has so far not been observed. The antheridia arise from the floors or the lower part of the walls of otherwise quite ordinary air chambers. Usually there is but one antheridium per chamber (Fig. 26), but that is not always the case, as shown in Fig. 28 and Fig. 24b, where an antheridial rudiment is developing behind the right (larger) antheridium separated by an incomplete septum, or inside a new secondary chamber. Some thalli have been seen in which almost every chamber in the front part of the thallus contained an antheridium. From the limited observations made so far, I judge that antheridium formation mainly occurs under short day conditions, while female organs seem to develop readily in the summer also. This may account for some of those thalli which seem to have female organs only. On the other hand, the antheridia are frequently very difficult to find. On numerous occasions they startled me with their presence in a preparation in which they had eluded me even during dissection.

The archegonia have six (or sometimes more) rows of neck cells and the usual four lid cells (Fig. 44). Even when unfertilized, old ones mostly have exceptionally fat bellies. They are borne on archegoniophores (the "involucres" of Carr) with carpocephala which are usually one (Fig. 26) or sometimes (Figs. 31, 32b) two sectored, i.e. contain one or two receptacles. Each receptacle contains from one to six archegonia. At the time archegonium primordia are recognizable as such (Fig. 24b) the archegoniophore may still be only a minute pad of tissue beneath; usually the carpocephalum is at least a saucer-shaped structure then. (I have so far not been able to sacrifice a sufficient number of specimens of this stage to give a conclusive account, especially in regard to apex, symmetry, and receptacular flaps, if any. There is some iindication that even a one-sectored carpocephalum, as is to be expected on theoretcal grounds, is not a radial structure during its early development.) The archegoniophore has a stalk of variable length (Figs. 30b, 33b, 35) which is radial and lacks any special features. As the carpocephalum expands, the tissue at the side of the receptacle enlarges also, until only the tips of the necks of the unfertilized archegonia project.

The carpocephalum contains air chambers opening by pores, which are initiated quite early. Their number varies. In some instances they are almost (Figs. 34a, b) or completely (Fig. 33b) lacking. Usually the pores are randomly but evenly scattered in the upper part. A single instance was observed of a carpocephalum with a circular depression around the receptacle, the pores opening neatly just inside the lower margin of the depression (Fig. 33a).

The large carpocephalum in the oldest segment of the plant shown in Fig. 36 showed heavy proliferation in its upper tissue. The cells were exceptionally coarse. No air chambers appeared to be present in the proliferation, which was deep green in color. But, as in the other carpocephala in my cultures, the occlusion of the receptacle, described by Carr (1956) as a routine post-fertilization change, did not take place. When dissected a fortnight after drawing, the receptacle contained five unfertilized archegonia.



FIGS. 37-46 — Fig. 37. Apex of thallus seen from above, showing conjugate apical cells and origin of air chambers (*a*). Figs. 38, 39. Barrel pores in l.s., from a razor handsection of a fresh carpocephalum. The pore in Fig. 39 is more mature (*k*). Figs. 40-43. Surface views of pores from carpocephala that had reached their full pre-fertilization development. Except Fig. 42, all from the same carpocephalum (*k*). Fig. 44. Open tip of archegonium from above. Four cover cells, the top tier of six neck cells below (*l*). Figs. 45, 46. Antheridia (*a*). (Fig. 37. Loc. 1, isotype; the rest loc. 2.)

The air chambers of the carpocephala open by barrel pores (Figs. 38, 39) of the simplest possible kind, being composed of but two tiers of cells. Most of the pores, in the unfertilized material examined by me, had only four cells in each of the outer and inner rings of cells (Figs. 40, 41), although pores with up to eight cells in the outer, and (counter to Carr's statement) also in the inner ring, were not uncommon (Figs. 42, 43). Figures 40-43 were deliberately drawn from mature thick-walled pores, and all but Fig. 42 from a single archegoniophore. A few pores showed a considerably wider external than internal opening, as described by Carr (1956). The pore canal is lined with the granular deposit classically referred

to as resin (Figs. 39, 41-43). It stains bright red with Sudan III dye.

The epidermis of the carpocephalum has a cuticle which readily takes up Sudan III.

The upfolding of the thallus margin reaches its greatest development around the archegoniophores, forming a pouch. The edge of the pouch frequently bears hair-like outgrowths (Fig. 27), but although the terminal cell of such a hair may be large and pale it does not appear to be glandular. The pouch tissue bears lamellae on its inside, seemingly representing walls of greatly distorted air chambers (Figs. 35, 29) and sometimes also a few hair or scale-like emergences. In one abnormal case (Fig. 32a) a double pouch was present. The normal inner pouch was separated from the outer by regular air chambers, and some lamellate outgrowths. A further abnormality is shown by the ventral sprout in Figs. 33a, b, which has an incomplete pouch.

In so far as the pouch is clearly composed of thallus wing tissue folded up around the apex of the plant, one would expect it to have a cleft at the front end. This is indeed sometimes the case (Fig. 27c, where the cleft extended to within a few cells of the origin of the archegoniophore stalk). But while the cleft is normally present in the early stages (Fig. 30b), later growth apparently is marginal or intercalary, so that most specimens finally show no trace of it (Figs. 33a, 35, 36). The growing point of the thallus is used up: further growth takes place sympodially by means of ventral sprouts. I believe that the archegoniophore is normally morphologically terminal, and that it, rather than the pouch, is responsible for using up the growing point. The ventral sprouts in Fig. 27c support this contention. On the other hand, abnormal specimens with other than terminal female organs have been seen. In Fig. 29 the hindmost archegoniophore, at least, cannot be terminal. Structurally, although it bore a normal archegonium, it is also simpler than the other two, being smaller and lacking air chambers. Figure 28 shows a single archegonium standing behind even a pair of antheridia. It appeared to have been the result of an extreme morphogenetic error, as it seemed to lack any trace of an archegoniophore. In an otherwise almost identical specimen such an archegonium stood on a rudimentary archegoniophore. A further plant bore, behind a terminal, developing archegoniophore, two apparently archegoniophore-free archegonia, widely spaced along the midline of the thallus in *Riccia*-style.

Many unsuccessful attempts have been made to induce fertilization. So far I have not discovered the necessary conditions. It is indeed extremely difficult, because of the surface tension effects created by the narrow diameter of the air chambers, to surround the antheridia with free water. The strange antheridial stalk suggests that perhaps some spectacular mechanism may be involved, such as the breaking off and ejection of the antheridial bodies. No such phenomenon has been observed, and I have previously been disappointed by the seeming lack of function of the extremely long and coiled antheridial stalks of the hornwort *Dendroceros*. A single pregnancy was, however, noted in an old and dirty soil culture. The embryo had aborted, presumably because the whole thallus was in an unhealthy condition.

Ventral Sprouts—The ventral sprouts mentioned above (Figs. 27, 33, 34, 36) are initiated quite early. They arise either from the solid tissue of the thallus beneath the air chamber layer, or from any place on the outer, ventral, surface of the protective pouch. The right first order ventral sprout in Fig. 36 has a second order sprout coming from barely below the pouch margin, and the small hump just behind it is also a ventral sprout rudiment. Sporelings may produce their sex organs early, but the precocity of ventral sprouts is much more extreme. Thus the left ventral sprout in Fig. 27c contained antheridia and a developing archegoniophore.

Regeneration — Any green vegetative cell appears capable of regeneration. Plants on very moist substrates and thalli disturbed by transfer give rise to regenerates all over their older surfaces. At the best such a regenerate starts with a cell comparable to a germ tube and under-

goes a course of development precisely like that of a normal germling, but frequently there is much irregularity.

ISOTYPE — The first germlings from locality 2 spores raised doubt as to whether they belonged to the plant described by Carr. Later came the realization that what had been illustrated by him was matched by the fertile tips of my plants. Dissection of dried plants from locality 2 confirmed a combination of the characteristic fertile parts with a rather more elaborate thallus, and the examination of Carr's (1956) photographs suggested that the type material also had rather "more" thallus than he claimed for it. When a locality 1 isotype soil sample became available, it was indeed possible to find air chambers in the dried thallus tissue.

During the sowing of isotype spores I noted their considerable ranges in color and size, but failed to make measurements at the time, not realizing that I was using up the best of the scanty material. Spore variation is largely between different capsules; individual capsules show spores of uniform color and almost uniform size. (This observation negates Carr's, 1956, p. 184, interpretation of progressive sporogenous tissue differentation with non-simultaneous meiosis. The general considerations set forth in Proskauer, 1954, p. 151-3, appear to apply here also.) Carr listed the spore size in the type material as 41·5 ± 5 μ in maximum diameter; my measurements on the isotype range from 39 to 51 μ, but I believe that I had at least one capsule with even larger spores among the sown material. Most of the capsules examined contained spores about 45 μ in size. In color the spores range from pale brown through reddish brown to pale wine red, but some blackish ones were included in the sowings. Color (a function of maturity) is unrelated to size (a function of capsule size and spore mother cell number). The spores appeared to be largely immature. The very few that germinated did so more slowly than locality 2 spores in parallel cultures. The capsules in the locality 2 field sample were consistently larger than those found in the isotype material, indicating better growing conditions, and consequently tended to contain larger spores (mostly about 60 μ in maximum diameter, range sampled: 53 to 63 μ). Also they were harvested at maturity (fide Carr, in litt.; and their color, contents and viability). The basic pattern of spore markings is identical in the two samples, but most of the isotype spores are less inflated and bear more delicate spines than the locality 2 spores. However, it was possible to find an almost exact match for certain larger locality 1 spores in those of a small-spored locality 2 capsule. I have a suspicion that the spore and spine size difference between the two samples may conceivably be accounted for in part by lack of deposition, or partial deposition only, of the outermost spore wall layer in the locality 1 plants. This will have to be tested on growing material.

A mere six isotype germlings were obtained. Although these were rather stunted in the early stages, probably because of the limited food reserves of the small spores, they followed the basic developmental pattern of locality 2 material and could be matched by individual sporelings. The surviving material constitutes a single clone. It behaves just like locality 2 material, and, on media *a* and *p* has yielded thalli as big and many-chambered as any of the latter. Just a few isotype plants have produced archegoniophores so far, and no antheridia have been seen; but since in this also there is agreement with simultaneous locality 2 cultures on the same media and photoperiods, there is no reason to doubt that the plants are monoecious.

Isotype clone material grown on medium *p* under a 10 hour day/14 hour night photoperiod has indeed proved to be monoecious and indistinguishable from locality 2 material. (Added in proof.)

I conclude that the isotype material is conspecific with the locality 2 material, but that in the field its growth was depauperate because of poor environmental conditions, and further that it was harvested prior to maturation of the spores.

Speculation

NATURE AND AFFINITIES OF *Carrpos*, AND SOME PROBLEMS ABOUT *Riccia "curtisii"*, ETC.— Only the scanty information

given by Carr (1956) on the environment in which *Carrpos* grows in the field is available to me. On the basis of this, and the peculiar culture media that plant flourishes in, I believe the following course of events probable. The spores germinate under water when the saltpan is leached and waterlogged by the winter rains. Later growth is subaerial, and the plants are subjected to progressively more saline conditions.

Carrpos is a member of the Marchantiales in the restricted sense (excluding the Sphaerocarpales). The different combinations of simple and complex features actually realized by the extant members of the order has forced on us the conclusion that the genera fall into a number of radiating series, which, with the possible exception of one of them, must perforce represent lines of reduction. *Carrpos* is a worthy addition to the team, in terms of the incongruity of the characters it combines. It even lacks in its thallus three of the general ordinal characters: it has no horizontal rhizoid system, it does not show the usual variety of rhizoid cell types, and it bears no ventral scales. In addition the thallus has expanded air chambers which are wide open at the top. But this is no cause for excitement. We do not even have to match things piecemeal. All these characters are similarly combined in the thalli of certain species of the subgenus *Ricciella* of *Riccia*, e.g. *R. crystallina* and *R. frostii*. (The reader who has not seen either of these plants alive is warned about the poor and often even erroneous descriptions of them in floristic works.) How striking this similarity is can be illustrated anecdotally. Two days ago my assistant, with a suspicious look on her face, pointed out some tiny plants that were developing on a raw soil culture. I told her that they belonged to *Riccia crystallina* and then learned that I had been suspected of having planted in jest some *Carrpos* between her *Athalamia*. Comparison, if less precise in detail, can also be made with the wide chambered juvenile phase of some of the more complex genera, and the adult thalli of *Stephensoniella* and *Aitchisoniella*.

The statement that *Carrpos* lacks ventral scales needs some qualification. The margins of the pouch surrounding an archegoniophore commonly bear teeth (Fig. 27) and then strongly recall the edges of certain ventral scales. Indeed, in the specimen illustrated by Fig. 30 the whole developing pouch structure resembles a pair of joined scales, but continuous with the thallus wing tissue. This could be built, via a comparison with the median riccioid scales, into a nice theory to explain why the pouch is usually closed in front. What I, however, believe we have here is another case of genes " misapplied " during morphogenesis, a frequent failing of the Marchantiales, which seems maliciously designed to ensnare those morphologists innocently clinging to a primitive concept of homology. " Misapplication " of scale genes is seemingly quite common: see the lacy structure representing the receptacular flaps in the *Marchantia* archegoniophore. In the case of *Carrpos* it should be remembered that the great majority of genera with archegoniophores have a precocious development and fertilization of archegonia, and use an entire investment of scales normally derived from the ventral surface of the carpocephalum, but occasionally in part misplaced on the dorsal surface of the thallus, to act as a water catching device during fertilization.

Carrpos shows its greatest degree of originality in its antheridia, both in their unique structure and detailed manner of disposition.

The archegoniophore is clearly the least reduced part of *Carrpos*. Its mere existence is a " primitive " feature according to the reduction hypothesis, as are the presence of barrel pores and the production of more than one archegonium per receptacle, by " receptacle " in the restricted sense being understood the individual fertile sectors of a carpocephalum, each equivalent to the entire unbranched female structure of a *Targionia* or *Cyathodium*. Measured against average archegoniophores, it is reduced because of the short stalk, the lack of scales and rhizoids, and the low number of receptacles. In the precise manner in which the receptacular tissue encloses the sporophyte it is uniquely specialized, unless *Aitchisoniella* (*see* below) or *Oxymitra* can be drawn into

comparison. (In trying to interpret *Oxymitra* one must now also bear in mind the additional complication presented by *Oxymitra cristata* Garside, 1958.) When reading Carr's paper, and long before I found that also in *Carrpos* more than one receptacle can actually be produced on a carpocephalum, my thoughts immediately turned to *Exormotheca* and Kashyap's (1914a, b; 1929; cf. Orth, 1929, for details of the ultimately widely open air chambers) *Stephensoniella* (cf. Mehra & Mehra, 1939) and *Aitchisoniella* (cf. Ahmad, 1938). Kashyap (1914a, p. 22) stated that the receptacle of *Aitchisoniella* opens by a narrow circular mouth, but the poor quality of both the published drawings and the exiccata in hand do not permit me to understand what goes on. But in some ways an even closer comparison can be made with *Athalamia* (*Clevea*) and *Plagiochasma*, genera believed to be in the same general series as the former three, but " higher up ", and on a somewhat specialized side branch. The specialization is expressed by the presence of but a single archegonium per receptacle, and the absence of a rhizoid groove in the archegoniophore stalk. This latter feature is correlated with the " misplacement " of the archegoniophores on the dorsal surface of the thallus, a phenomenon that is routine here (for details and intermediates *see* Bergdolt, 1926; and Goebel, 1930) and happens in *Carrpos* occasionally.

Some living unfertilized archegoniophores of *Athalamia* (*Clevea hyalina*) I just examined looked exactly like that of *Carrpos* in Fig. 31 but for the air chambers. In *Athalamia* all the air chambers that are present open, as in *Targionia*, in the manner supported by theory if not reason, on the inside (the morphological upper side) of the receptacular flaps[2]. In *Athalamia* at maturity these chambers are wide open (cf. also Kashyap, 1929), and the inside of a fully grown receptacular flap or valve is strongly reminiscent of the inside of the thallus pouch surrounding the *Carrpos* archegoniophore. *Plagiochasma* archegoniophores resemble those of *Athalamia*, but when air chambers are present at all in the carpocephalum, they lie in the tissue between the receptacles and open to the outside by barrel pores (Kashyap, 1929; Orth, 1929).

The sporophyte of *Carrpos* is at precisely the same level of organization as that of *Corsinia*. It differs in often having a longer seta (frequently longer than described by Carr, but never uniseriate as in *Riella*).

The spores of *Carrpos* at first sight look like small versions of those of the sphaerocarp *Riella* (Proskauer, 1955). But on squashing, the spores of these two genera behave quite differently. In mature spores of *Carrpos* (locality 2) two separate colored outer layers are readily removed from the inner colorless one.

In the Marchantiales the spores (cf. Inoue, 1960, for some illustrations) of the complex genera around *Marchantia* stand apart by their structure and small size. The bulk of the genera have " blister " spores, in which the outer coat is raised in part into hollow bubbles. Simple spines are found in most species of *Cyathodium* (*C. spruceanum* Proskauer, 1951, acts as a connecting link), but they differ from those of *Carrpos*. The spores of *Ricciocarpos* show some semblance to those of our plant. Spore markings have never been satisfactorily exploited in assessing relationships in the group. Speculation is futile until we know in which spores reticula and spines represent uninflated, deflated, or broken " blisters ". The " palynological " approach is quite unhelpful, but in the ontogenetic studies we appear to have progressed very little since Leitgeb's (1884) survey. It turns out that the closest match to the spore markings of *Carrpos* is in the spines of the membrane which covers the persistent spore tetrads of *Riccia curtisii* auct., sensu lato, especially those of immature spores of the " Texas " (*see* below) plant.

The germ tube of *Carrpos* issues from the outer face of the spore. I have pre-

2. In *Athalamia* and some other marchantioids the outer surfaces of carpocephalum and receptacular flaps are smoothly continuous. Possibly in these cases not only the inner but also the outer surfaces of the flaps are " upper " side tissue. Where air chambers are present in such receptacular flaps, the plants frequently manage to have most of the pores on the exposed outer side (e.g. *Asterella californica*; *Neohodgsonia mirabilis*, New Zealand material checked).

viously stated (Proskauer, 1955, p. 70) that such behavior implies an ancestry of forms with permanently united tetrads. Application of this dictum to the Marchantiales was begun, at my prompting, by Inoue (1960). Besides *Carrpos*, and possibly *Stephensoniella* (Mehra & Kachroo, 1952), the entire genus *Riccia* (review in Inoue, 1960) is now believed to show outer face germination. Consequently, we should re-examine in a fresh light those species which preserve the presumed ancestral condition for these genera of permanently united spores, namely *Riccia curtisii* and its allies. They are usually included in the subgenus *Ricciella*, although some assign them to a third subgroup or even separate genus, *Thallocarpus*. The air chambers are of the, at maturity wide open, *Riccia crystallina-Carrpos* type. Peg rhizoids and rudimentary scales may be present (cf. Schiffner, 1936). The bulk of the many investigated species of *Riccia* have 8 chromosomes (7 + m) as the basic number, although the numbers 9 and 10 have demonstrated in some (cf. Berrie, 1960). *Riccia curtisii* sensu lato also has 8 chromosomes, and in the plant from Florida these consist of 7 autosomes and a heterochromatic sex chromosome, minute in the male, much larger than any other in the female (Lorbeer, 1934; Siler, 1934, *see* figures — not text !)[3]. In this peculiar chromosome set we have a close parallel to that of *Sphaerocarpos* (except *S. cristatus*). The *Sphaerocarpos* set is presumably derived from a typical liverwort 8 + m set as found in *Riella* (cf. the scheme of Tatuno, 1959). I suspect that the *Riccia curtisii* set similarly derived from the ancestral 8 + m set maintained in some *Ricciae*, and in turn yielded the 7 + m set of most other members of the genus. I have been aware of the problem for some years and suspect that a fairly simple mechanism accounted for a parallel change in these cases. I have previously drawn attention to the fact that the m chromosome in 8 + m monoecious *Riella* plants corresponds to the y chromosome in 8 + y males of dioecious *Riella* (Proskauer, 1955). The best theory I have is as follows: The m chromosome is adjusted to contain genetic material relevant in the formation of male sex organs. One of the autosomes, by interchange, loses such somatic genes that are not also present on the m, and picks up heterochromatic material from the other chromosomes. This might happen during the interphasic " bouqueting " of heterochromatin. The set is now composed of 7 + M + m. By failure of division (either in the haplophase, cf. sectoring in *Marchantia* and *Sphaerocarpos*, or during meiosis II), 7 + M and 7 + m cells are produced and are viable. This explanation is not in conflict with the actual cytological and genetic findings. The chromosome situation of *Carrpos* I hope to discuss in a later paper.

To return to the systematic position of *Carrpos*. I believe that it is an offshoot of a " pre-Riccia " pool in the reduction series, bounded at the upper end by the more complex members of the Cleveaceae and the closely related Rebouliaceae. The plants in this pool, by neoteny, ela-

3. These reliable findings are in sharp contrast with the reasonably convincing illustrations of 7 + m chromosomes in both the male and female thalli of Texan specimens by McAllister (1928). I examined material in Herb. UC from Sanford, Florida (leg. Rapp, 12. v. 1911, and iv. 1931) and Austin, Texas (leg. Young, 10. ii. 1914). Minor but obvious differences in the spores, coupled with vegetative differences in rhizoids, etc., suggested to me that two related but discrete entities are involved. Later I noticed that Duthie & Garside (1936, 1939), examining material from the same localities, had arrived at an identical conclusion. According to them the Texas plant agrees with the type material (from North Carolina), but this requires confirmation. Schiffner (1936; for Fig. d-m, Texas, read Florida) also realized that two plants are involved, and distinguished, conditionally and invalidly, the forms *tenuis* and *crassior*. This is unsound, for our two Sanford, Florida, samples are respectively delicate and robust, but agree otherwise. His comments on Stephani's treatment caused me to observe that, unless a combination *Riccia curtisii* (Austin) can be found antedating the name *Riccia curtisii* Stephani, both components of our plant need a new name under the genus *Riccia*, as Stephani did not accept the basionyms and seems to have described afresh from a totally different plant (but see Stephani, Icones ined., in Herb. UC, sub *Riccia crystallina* !). Probably some of the synsporous *Ricciae* described from South America, South Africa, and India may take care of part of the nomenclatural problem, but this mess needs cleaning up.

borated the wide chamber type juvenile thallus. They bore antheridia in circumscribed groups along the midrib, but still maintained archegoniophores. Included were forms with spores united in tetrads. *Carrpos* became differentiated by simplification in the lower surface appendages, the dispersal of the antheridia, and some reduction and elaboration in the archegoniophore. The sporophyte also underwent some reduction, and the spores separated again.

Because of the current tendency to draw family limits rather finely, it best stands in a family of its own, the Carrpaceae.

Carrpaceae, familia nova Marchantialum.

Monocarpaceae Carr, 1956, p. 187, nomen nudum.

Cavernae propter aërem thallorum foraminibus latis, carpocephalorum tectis poris compositis. Sporogonium pede setaque. Cellulae steriles capsularum simplices.

Thallus with wide open air chambers. Carpocephalum with roofed air chambers with barrel pores. Sporophyte possessing foot and seta. Capsular sterile cells simple.

Typus: *Carrpos* Proskauer, 1961b.

The information that *Carrpos* occurred even in Western Australia (Carr, in litt.) enhanced the possibility that the plant had previously been collected and described under a different name. Over a century ago Preiss collected many liverworts in Western Australia, and Drummond literally must have scoured the "Swan River" area searching for minute ones. I have glanced at the many *Ricciae* of Drummond described by Taylor (isotypes in Hb. Mitten in Hb. NY) but they do not include our plant.

The Nature of the Marchantialean Air Chamber — There is now agreement that the air chambers of the Marchantiales originate by minute holes being formed schizogenously at the point of contact of, typically four, epidermal cells lying just behind the apex. From this uniform origin the different kinds of mature chambers develop through division and enlargement of the lateral and basal cells confining the hole. It is the precise localization and direction of these further changes which accounts for the differences in basic shape. The number of chambers per segment of the thallus depends on the timing of hole formation. If hole formation occurs early in the differentiation of a segment of the thallus, that segment will have few air chambers. If it occurs after appreciable subdivision has taken place, and holes are formed between all the epidermal cells of the segment existing at that time, the segment will contain many chambers. (For illustration of the rather complicated processes involved see Menge, 1930, on *Plagiochasma* germlings, and Burgeff, 1943, on *Marchantia*.) Two kinds of symmetry of the adult marchantialean thallus exist: the *Marchantia* type, in which there are two lateral rows of metamers on both the upper and the lower side from the beginning, and the *Riccia* type, in which the upper surface is bilateral, and the lower side has a single row of metamers, the latter frequently marked by scales initiated in a single row, but subject to later tearing to yield two parallel series. The simplest example of the *Marchantia* type is found in my *Cyathodium spruceanum* which has two rows of air chambers and two rows of ventral scales (Proskauer, 1951; 1961a, Fig. 1: 2). Here the scales mark the individual lower surface segments, but there is some evidence to suggest that each surface segment corresponds to two half air chambers, the segment limits running through the centre of each pore, as shown by some segments of Menge's (1930) *Plagiochasma* germlings.

Recently Mehra (1957a, b) suggested that the Marchantiales had their origin "from foliose Jungermanniales through a stage analogous to that of *Petalophyllum.*" It has long been known that in *Petalophyllum* there is a congenital fusion of lateral leaves (like those of its close relative *Fossombronia*) in such a way that roughly half of each leaf contributes to the flat wing tissue of the thalllus while the remainder of each leaf appears to be inserted on this wing like a lamella. This wing with its lamellae Mehra equated with the floor and (some of) the side walls of the marchantialean chamber, leaving the roofing to later refinement. A much better case could have been made for such a theory if the sphaerocarp *Geothallus*

had been invoked instead of *Petalophyllum*. *Geothallus* belongs to the same series of liverworts as the Marchantiales, not to the series with specialized premeiotically four-lobed spore mother cells (cf. Proskauer, 1961a). Further, it not only shows all the forms of leaf fusion seen in *Petalophyllum* (but see the original diagnosis of *P. preissii* Gottsche), but there are many elaborations, such as the formation of a network of low chambers over the midrib region resulting from the fusion of upper surface outgrowths (Doyle, 1962). Be that as it may, as I pointed out to Professor Mehra when he showed me the papers prior to their publication, the theory completely disregards, and is incompatible with, the ontogeny of the marchantialean chamber, where in the beginning there *is* a hole, and not the upgrowth of tissue from an uninterrupted solid surface.

The sporeling of Marchantiales, like that of *Sphaerocarpos*, *Anthoceros* L. emend. Prosk., *Lycopodium* L. emend. Rothmaler, *Equisetum* sp., etc., is of the obconical "golf tee" type. Inspired by the writings of the late Professor F. O. Bower, and the theories of the late Professor Kashyap, I have previously commented on this "golf tee" as follows (Proskauer, 1954, p. 146) — "The first stages in the development conform to what will probably in due course become recognized as the basic land plant gametophyte pattern: a radial upright obconical structure is formed, which acquires a depression at the top." In its early stage the sporeling of *Carrpos* displays a golf tee structure comparable to that found in most other Marchantiales (cf. Inoue, 1960). Then the first air chamber expands, and the top of the original golf tee is pushed aside as the tail. But the whole still has the appearance of a golf tee germling, the first air chamber forming the new top part (cf. Fig. 21 with Fig. 1 of Proskauer, 1954). Could this be more than a mere matter of chance appearance, and in fact an ontogenetic repeat? In this case the air chamber is an expansion of a depressed golf tee top homologue. Each further air chamber is then a further similar repeat (cf. Figs. 24a, 25a).

This theory hinges on the nature of the original golf tee top. It is indeed generally a quadrant of cells at the beginning. But is a hole formed between these cells? This is exceedingly difficult to judge, largely because the cells are not confined laterally in a tissue. The manner in which they ultimately are spread apart (Figs. 8A, 18, 19, 22) is suggestive of their being in fact a separation of these cells at their common contact point. In this case the tail would be the potential first chamber, but in *Carrpos* it fails to expand properly, perhaps because of morphogenetic difficulties related to cell size.

The golf tee top should be re-examined in a new light, and not just in the Marchantiales. I have looked at many accounts of sporelings of the latter group, and these have underlined the unique quality of the paper by Menge (1930). But even from his precise drawings of *Plagiochasma* the possibility that the quadrant cells separate during their enlargement cannot be proven or disproven. In any case, as in the majority of Marchantiales, the quadrant gives rise to a concave disc (the typical depressed golf tee top). At one side of its rim the growing point is organized, the air chambers are formed from holes within the concave disc. If the depressed golf tee top is indeed the first shallow air chamber, then all further chambers, strictly speaking, would be equivalent to lateral or secondary chambers (cf. Orth, 1929). In *Carrpos*, because of the rapid expansion of air chambers in the proximity of the growing point, it is often difficult to judge whether a new chamber is initiated just on the inside or just on the outside of the rim of the next older one.

The theory here proposed of the nature of the marchantialean air chamber satisfactorily answers the questions that I personally have had. According to it, for the air chamber, perhaps the most striking single character of the order, the plants are indebted only to their own germling. The scale then is a structure unrelated to the air chamber, being formed as an outgrowth of the outer parts of a segment, just like the leaves of Jungermanniales, Bryidae, etc. It may be an ancestral feature here; equally well it may have been invented de novo.

Summary

Carrpos (olim *Monocarpus* Carr), a liverwort from saltpans in Australia, was studied in culture. Successful spore germination was observed only in submersed culture and on highly alkaline soils. The structure and development of sporeling and adult thallus are described. The thallus proved to be more complex than was indicated in the original description, being comparable to that of *Riccia crystallina*. The plant is monoecious. The antheridia have uniseriate filamentous stalks. They are developed inside thallus air chambers, which are wide open exactly like the vegetative ones. On the other hand, the archegonia are still borne on archegoniophores whose carpocephalar air chambers have two-tiered barrel pores.

Carrpos is considered to be an offshoot of a "pre-*Riccia*" pool in a reduction series in the Marchantiales and is placed, by itself, in the new family *Carrpaceae*. It is believed that this "pre-*Riccia*" pool included forms with permanently united spore tetrads, a character still present in *Riccia curtisii* auct. and its allies. A way of arriving at the karyotype of most members of the genus *Riccia* through that of *Riccia curtisii* pro parte is suggested. *Riccia "curtisii"* is in urgent need of taxonomic and nomenclatural revision.

The structure of the sporeling of *Carrpos* inspired the theory that the air chambers of the Marchantiales are elaborations and ontogenetic repeats of the concave top of an obconical "golf tee" germling.

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THE FUNCTION OF THE GLUME PIT AND THE CONTROL OF CLEISTOGAMY IN *BOTHRIOCHLOA DECIPIENS* (HACK.) C. E. HUBBARD

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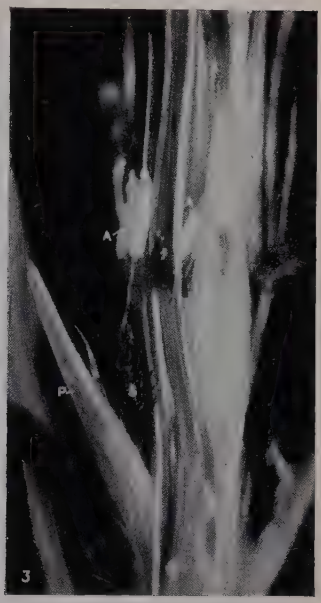
Bothriochloa decipiens (Hack.) C. E. Hubbard, of the tribe Andropogoneae, is an endemic Australian grass related to the Indian *B. pertusa* (L.) A. Camus. According to Blake (1944) it is very abundant in the Eucalyptus forests of Queensland, particularly towards the south and east, extending into northern New South Wales. In its natural area it is very frequently cleistogamous, and in cultivation in America it is reported as habitually so (Celarier, 1958, private communication). Experiments at Belfast during 1959 and 1960 showed that the cleistogamy of the species is environmentally controlled, especially by photoperiod.

Species of *Bothriochloa*, in common with others of the subtribe Andropogonineae, possess digitate or paniculate inflorescences with the spikelets borne in pairs, one pedicellate and the other sessile. In *B. decipiens*, the pedicellate spikelet is severely reduced, consisting merely of the inrolled lower glume. Of the two florets in the sessile spikelet the lower is represented by an empty lemma; the upper is hermaphrodite and fertile, being thus the only functional floret in the nodal group. The floret is itself unusual in possessing only a single stamen. This characteristic, unique in the genus, may be regarded as an adaptation associated with cleistogamy, but the floret is otherwise structurally normal; the lodicules are not especially reduced, as is commonly found in habitually cleistogamous grasses (Uphof, 1938).

The lower glume of the sessile spikelet bears a deep circular depression or pit, about one-third of its length from the tip. This feature appears also in other species of the genus, notably *B. ambigua*, *B. ewartiana* and *B. intermedia*. However, while pits are almost invariably present in the glumes of *B. decipiens*, in other species the pitting is rather inconstant. Pitted and unpitted forms may occur together,



FIGS. 1-3 — (*A*, anther; *S*, stigma; *P*, glume pit.) Fig. 1. Chasmogamous inflorescence of *Bothriochloa decipiens*, formed under long days. Fig. 2. Chasmogamous floret, shortly after the dehiscence of the anther. The glume pit is clearly discernible. Fig. 3. Two chasmogamous florets of *B. decipiens*. In that on the left the pollen is shed and the glume is closing. In that on the right, the glume has closed, entrapping the anther, but above the level of the glume pit. Fig. 1, × *c.* 1·5; Figs. 2 & 3, × *c.* 4·5.

and pit development may even vary within the same inflorescence (Blake, 1944).

Although the significance of the glume pit has always been something of an enigma, it is hardly reasonable to suppose that so conspicuous and unusual a feature should be without a function of some sort. Study of flowering behaviour in diverse environments shows that it does in fact play a key role in the mechanism of cleistogamy, at least in *B. decipiens*. Its function is that of an obturator, preventing the emergence of the single anther from the floret and ensuring its dehiscence in contact with the stigmas in all florets where the glumes do not open normally at anthesis.

The position of the glume pit, or, more cogently, the complementary boss on the inner side of the glume, is critical in permitting this function. In the young floret before pollination it depresses the lemma between the two branches of the stigma, lying just above the level of the tip of the immature anther. Should the glumes open at the maturation of the floret, normal chasmogamous anthesis is possible, since there is no barrier to the exsertion of either stigmas or anther. Should the glumes fail to open when anthesis would normally occur, emergence of the stigmas is prevented by their engagement upon the depressed lemma below the boss. As the filament of the stamen extends, the anther is then driven hard up against the boss, and internal dehiscence takes place at the tip in contact with the stigmas.

Chasmogamous flowering is illustrated in Figs. 1-3 in an inflorescence of a plant grown throughout its life in a daylength of 18 hrs. or more. In this daylength with an air temperature of 22·5°C and a relative humidity of 75 per cent, anthesis most commonly occurs in the early evening, some four hours before the end of the photoperiod. The florets of any one inflorescence mature somewhat irregularly,

FIGS. 4-7 — (*A*, anther; *S*, stigma; *P*, glume pit; *G*, ovary; *D*, depression produced by the glume pit.) Fig. 4. Cleistogamous inflorescence of *B. decipiens*, formed under short days. The sheath has been opened slightly to expose the florets, in most of which caryopses are already forming. Fig. 5. Single spikelet of a cleistogamous inflorescence, with the glume folded back to show the position of the glume pit in relation to the stigmas. Fig. 6. The same floret as in Fig. 5, with the stigmas pulled aside to reveal the position of the dehisced anther in relation to the glume pit. Fig. 7. Longitudinal section of a cleistogamous floret to show the role of the glume pit as an obturator. Fig. 4, × *c.* 1·5; Figs. 5-7, × *c.* 4·5.

and flowering in the various branches may be spread out over some days, without any marked succession. In a chasmogamous floret, the glumes open gradually over a period of 10-15 min., and the stigmas and the undehisced anther emerge 8-10 min. after the first movement. Dehiscence takes place rather suddenly, 15-20 min. after exsertion (Fig. 2). The glumes close again after 50-70 min., sometimes entrapping the anther (Fig. 3). Even when this has happened the fact that chasmogamous flowering has taken place is revealed by the position of the anther in the floret, always *above* the level of the glume pit.

A cleistogamous inflorescence is illustrated in Fig. 4. and dissections to show the role of the glume pit in cleistogamy in Figs. 5-7. The removal of the glume from a floret of an old cleistogamous flower reveals the tangled stigmas, depressed centrally below the position of the pit (Fig. 5); on pushing apart the stigmas, the depression is seen to lie over the upper part of the anther (Fig. 6). In section, the dehiscence of the anther is clearly seen to have been just at this point (Fig. 7). The filament of the stamen in cleistogamous florets lies entangled at the rear of the caryopsis; normally it elongates to the same extent as in the chasmogamous floret.

There appears to be little or no difference in the size of functional pollen grains in chasmogamous and cleistogamous florets, although in the conditions in which cleistogamy is habitual pollen fertility appears to be somewhat lower. Pollen germination in cleistogamous florets takes place after the extension of the filament; only those grains near the point of dehiscence seem generally to germinate, but occasionally in sectioned anthers pollen tubes may be seen at all levels to the base of the thecae.

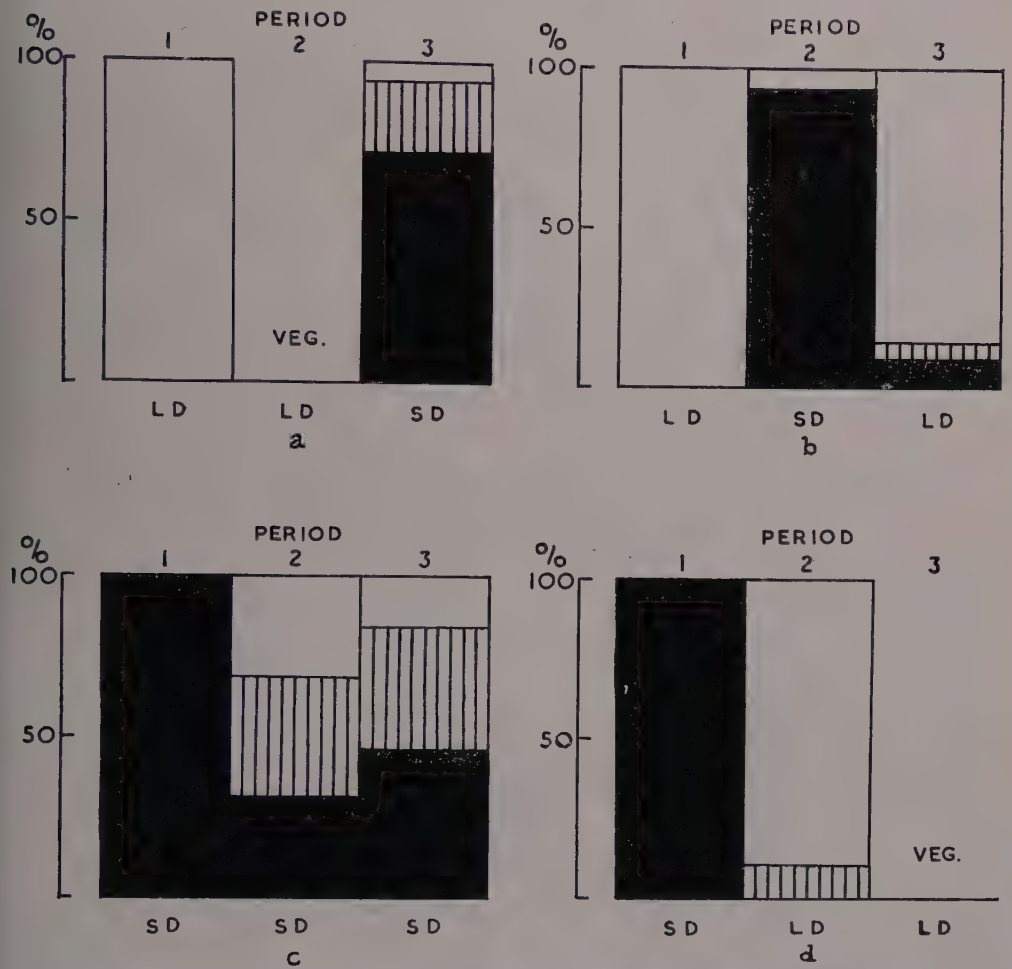


FIG. 8 — Flowering behaviour of pairs of plants in three successive periods of eight weeks under different photoperiodic regimes. *LD*, daylength>18 hr.; *SD*, daylength=8 hr. Proportions of inflorescences formed shown in three categories: black shading, cleistogamous; vertical hatching, partly cleistogamous; unshaded, chasmogamous. Veg., plants vegetative. Temperature governed above 22°C. throughout. Plants watered to run-off daily, and relative humidity maintained c. 75 per cent.

The function of the glume pit being as it is, whether or not cleistogamy occurs in any particular floret clearly depends upon the constraint placed on the opening of the glumes of the spikelet. A common cause of constraint is the failure of the inflorescence to emerge from the subtending leaf sheath; no pollen is then shed, and the entire inflorescence is cleistogamous (Fig. 4). Whether the inflorescence is exserted or retained in the sheath is determined by various environmental influences, and in this manner versatility in breeding system can apparently be attained.

Among the controlling factors, photoperiod can be shown to play an important role, at least in experiment. In flowering response *B. decipiens* seems to be more or less daylength-indifferent, since inflorescences are differentiated in both long (18 hour or more) and short (8 hour) days. The emergence of inflorescences from their subtending sheaths, however, varies in

different photoperiodic regimes. Some characteristic responses are summarized in Fig. 8 in terms of cleistogamous, partly cleistogamous and wholly chasmogamous inflorescences formed in three successive 8-week periods after the onset of flowering. Plants grown under long days underwent a period of vigorous flowering, during which they were wholly chasmogamous; they then passed into a period of vegetative growth. In a second period of flowering under short days, the same plants produced a high proportion of cleistogamous inflorescences (Fig. 8a). When plants were transferred from the long day environment before the end of the first flowering phase, the production of new inflorescences continued without a period of vegetative growth, and again the majority was cleistogamous. Subsequent restoration to long days induced a reversion to chasmogamy (Fig. 8b). Plants under continuous short days were wholly cleistogamous during the first eight weeks of flowering, and then showed a shift to partial chasmogamy during sixteen further weeks of flowering (Fig. 8c). Those transferred to long days after eight weeks of flowering in short days switched immediately to a higher level of chasmogamy, and then reverted to vegetative growth (Fig. 8d).

The emergence of the inflorescence depends, of course, on the relative growth of axis and subtending sheath, and it is of interest to determine which it is, axis or sheath, that is affected by photoperiod. The mean dimensions of the axis, measured from the uppermost node to the first basal branch of the inflorescence, and the sheath, measured from the node to the base of the lamina, are given in Table 1 for inflorescences formed in two photoperiodic regimes. It is evident that the variation in sheath length is slight but that the extension of the axis is related to the prevailing photoperiod. It may be concluded that this is the morphological variable determining whether or not a particular inflorescence will remain obligatorily cleistogamous or emerge and become at least potentially chasmogamous.

Although the conditions of these photoperiodic experiments are not directly comparable with any the plant is likely to encounter in its natural area, the attested occurrence of both cleistogamy and chasmogamy in wild populations does suggest that the breeding system is versatile in nature. Obviously other factors could be concerned with the natural regulation of stem extension growth during flowering; unfavourable temperatures and temporary drought are two other possible agents of control. It is noteworthy that the experiments of Brown (1952) have shown the availability of soil moisture to affect cleistogamy in *Stipa leucotricha* (although through a different mechanism from that in *Bothriochloa decipiens*) while Harlan (1945) has attributed the formation of cleistogamous panicles in *Bromus carinatus* to generally unfavourable environmental conditions during a flowering phase.

TABLE 1 — MEAN LENGTHS OF INFLORESCENCE AXIS AND OF SUBTENDING LEAF SHEATH IN SINGLE PLANTS OF *BOTHRIOCHLOA DECIPIENS* FLOWERING UNDER LONG (>18 HR) AND SHORT (8 HR) DAYS

Daylength	Length of inflorescence axis cm	Length of subtending sheath cm
LD[1]	19·36±1·07	9·62±0·53
SD[2]	6·58±0·54	9·78±0·50

1. A plant of the group of Fig. 1b, scored in the third flowering period. Sixteen inflorescences.
2. A plant of the group of Fig. 1a, scored in the third flowering period. Eight inflorescences.

As Fryxell (1959) has pointed out, autogamy is certainly no evolutionary *cul de sac*, provided that it is facultative. It seems that *Bothriochloa decipiens* may provide an example of a species which has achieved a compromise between potentialities for in- and outbreeding subject to environmental control, the mechanism depending upon a few minor morphological adaptations coupled with a marked sensitivity of flowering-stem growth rate to external influence. Experiments with other species of *Bothriochloa*, including *B. pertusa*, and with sexual species of the neighbouring genus, *Dichanthium*, have shown that photoperiodic control of stem extension growth may here also influence

the breeding system by causing intravaginal anthesis, although the refinement of the glume pit is lacking. Photoperiodic regulation of in- and outbreeding has also been demonstrated in *Rottboellia exaltata* (Heslop-Harrison, 1959). It is tempting to conclude that facultative cleistogamy is a common feature in tropical and subtropical Andropogoneae. The elucidation of its significance in terms of ecological adaptation must await a wider extension of genecological study of the kind already lavished on temperate floras.

Summary

Bothriochloa decipiens (Hack.) C. E. Hubbard is an Australian endemic grass of the tribe Andropogoneae which is commonly cleistogamous in nature. There is one functional hermaphrodite flower at each node of the inflorescence, containing a single stamen. The glume of the fertile spikelet bears a deep depression, the *glume pit*, which acts as an obturator in cleistogamous flowers, preventing the exsertion of the anther and causing its dehiscence in contact with the stigmas. Cleistogamy commonly results from the retention of an inflorescence within its subtending sheath; in these conditions, the glumes cannot open at anthesis, and the function of the glume pit as an obturator is fulfilled. If the inflorescence emerges, the glumes are free to open and the florets may then be chasmogamous. The extension growth of the inflorescence axis, which determines whether or not an inflorescence will be exserted, is related to prevailing daylength; under short-day conditions it is slight and a high level of cleistogamy is established; in long days, greater extension growth permits chasmogamous flowering. The mechanism may provide the basis for an environmental regulation of the breeding system.

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NODAL ANATOMY OF *BOERHAAVIA DIFFUSA* L.*

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Introduction

Anatomical anomalies are well known in many genera of the family Nyctaginaceae. Among such forms *Boerhaavia diffusa* L. is one of the most widely distributed indigenous plants. The anatomy of its internode has been studied in detail by Maheshwari (1930), while the petiolar structure was described by Bhargava (1932). Mitra & Bose (1957) have figured a transverse section through a node but otherwise the anatomy of the node has remained undescribed. The present investigation was, therefore, taken up to fill this gap (*see* also Pant & Mehra, 1961). In this connection it is worthy of note that according to Bailey (1956) more emphasis should be placed on the tracing down of vascular strands of leaves into the nodal region or further down in the stem for future determinations of phylogenetic relationships. While studying the anatomy of the stems it was found that in spite of the very common occurrence of this plant, even some of its obvious external features have not been mentioned by previous workers. These are also described in the present paper.

Material and Methods

The material was collected locally, during different seasons of the year and fixed in formalin-acetic-alcohol. Both hand-cut and microtomed serial sections were examined. Longitudinal sections were cut both in the plane of the two leaf bases and at right angles to it (*see* Fig. 13B-I). In addition, whole mount transparencies of the stems and leaves were prepared by Debenham's method (1939). Attempts were made to dissect out the vascular strands from the nodal region of the stems after softening the material in five per cent NaOH. But the presence of a complete cylinder of hard tissue (belated ring of vascular bundles and conjunctive tissue) materially obstructs a successful separation of the strands from inside a mature node.

Observations

External Morphology — The plants of *Boerhaavia diffusa* in their vegetative phase show nodes which have, besides the axillary branch, usually one or two accessory branches in the axil of either of the two leaves of a node (Fig. 1A, B). The branches in the axil of the smaller leaf are always better developed. During the flowering phase the stems develop an additional (extra axillary) branch somewhat laterally to the petiole in the interpetiolar region of the parent axis. This branch develops only on the side of the small leaf, but never on the side of the larger leaf (Fig. 1C). After the maturation of the fruits the extra axillary branch falls off due to the formation of an abscission layer (Fig. 7D) at a level slightly above its base.

Anisophylly, both in size as well as form, occurs (*see* also Engler & Prantl, 1934). The opposite leaves of each node are characteristically unequal and they alternate in successive nodes (Fig. 1B, C). The apices of leaves are sporadically variable, some are comparatively pointed while others are more or less obtuse or emarginate (Fig. 1D-H). Practically all leaves show asymmetry on the two sides of the midrib. Pairs of leaves belonging to fifty nodes were measured at one-fourth, half and three-fourths of the total leaf length to study the asymmetry of the leaves on either side of the midrib (*see* Table 1, wherein measurements of leaves

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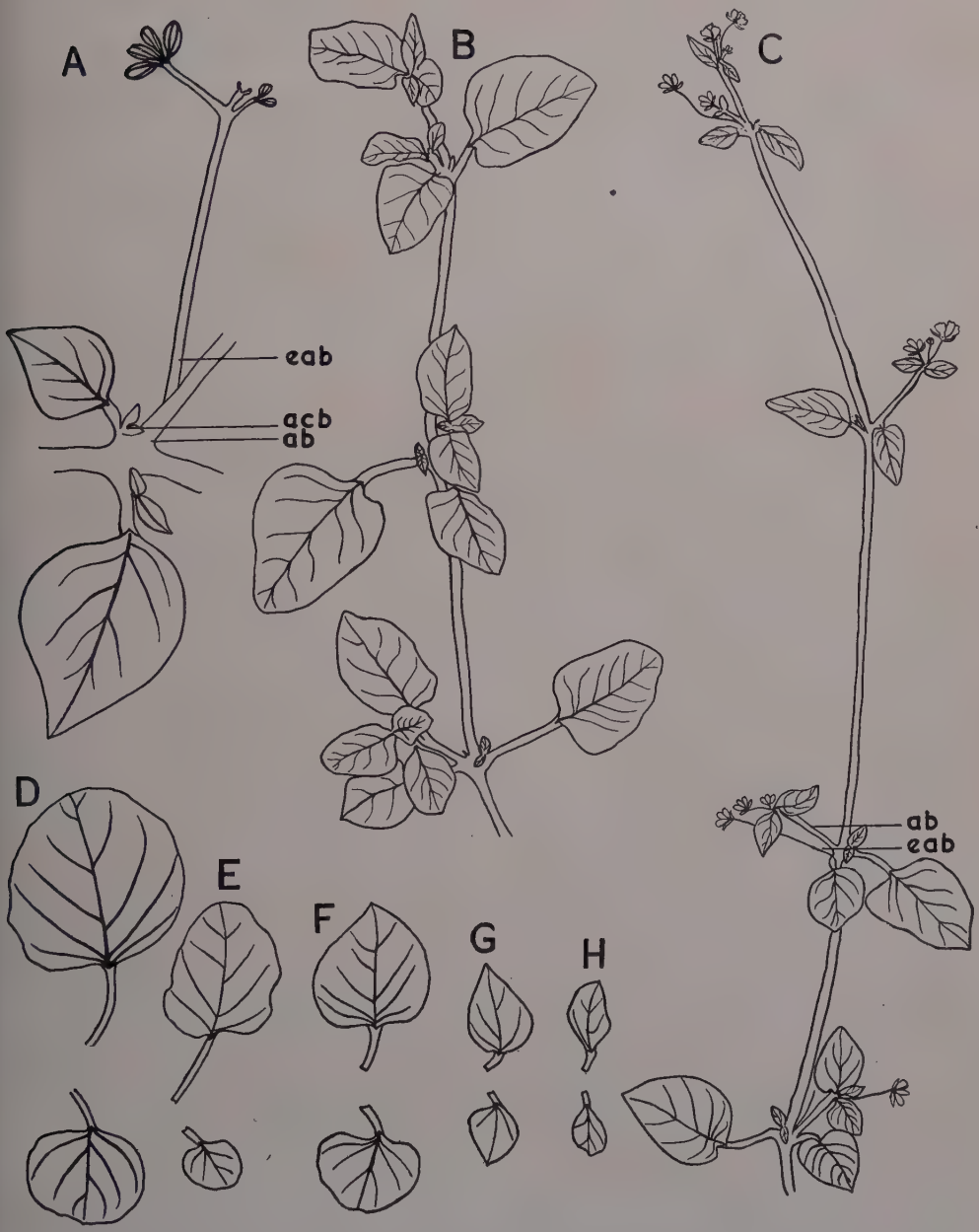

FIG. 1A-H — External features of shoots and leaves. (*ab*, axillary branch; *acb*, accessory branch; *eab*, extra-axillary branch.) A. Node showing accessory branch, base of axillary branch and extra-axillary branch. × $\frac{3}{2}$. B. Vegetative shoot showing anisophylly of leaves and asymmetry on the two sides of the midrib. × $\frac{1}{2}$. C. Fertile shoot showing axillary and extra-axillary branches, the latter are always borne on the side of the smaller leaves. × 1. D-H. Pairs of leaves from individual nodes of different plants showing anisophylly and also variation in form of leaves. The apex of the lower leaf in F is emarginate, others are obtuse or obtusely pointed. × $\frac{1}{2}$.

of only 12 nodes are given). The measurements of leaves in different nodes of the same shoot and of nodes in similar or different stages of development in different shoots show no correlation between the amount of asymmetry on the two sides of the midrib and the leaf length, or the relative age of the node. The smaller segment of most leaves lies towards the apex.

The stomatal index which, according to Salisbury (1928), is relatively more constant for a plant species than the number of stomata per unit area, was determined as 14·5 for lower epidermis and for upper 14·6 (in plants growing in the same light intensity). The orientation of raphidal bundles of the leaf is in two directions, most of them specially those near veins lie parallel to the plane of flattening but others are placed at right angles to this plane. Such a two-directional orientation of raphides must necessarily support the mechanical system of the leaf.

Anatomy of the Internode — According to Maheshwari (1930) the internode has two centrally situated large medullary bundles, these are surrounded by a ring of loosely arranged and very unequal bundles and the latter, in their turn, are followed on the outside by a well marked third ring called the belated ring of bundles, which consists of vascular bundles joined by lignified thick-walled conjunctive tissue (Fig. 13A). The various types of xylem elements and conjunctive tissue cells are shown in figures 10B-I. The belated ring is surrounded by a ring of one to two layers of thin-walled cells (termed " pericycle " by Maheshwari, 1930) outside which is a more or less clearly differentiated layer of somewhat thick-walled endodermoid cells (Esau, 1953). Most of the cortex is parenchymatous, its outer layers being chlorenchymatous; the hypodermal layers consist of collenchyma cells. The epidermis is single-layered and has glandular hairs and stomata.

Anatomy of the Node — The cortex endodermoid layer and " pericycle " of the node are similar to those in the internode. The characteristic arrangement of the vascular bundles of the internode, however, changes in the node.

Course of the Medullary Bundles through the Node — The two medullary bundles of an internode are rather like two flat ribbons lying parallel to each other (Fig. 13A). On approaching the node, where the branches on either side of the parent axis arise at appreciably differing levels, each of the two flat medullary bundles first of all gives rise to a small lateral strand on the side of the lower branch, which develops in the axil of the smaller leaf (Fig. 2A). These lateral strands form the two traces of the lower branch in their later course. In other cases where the vertical distance between the levels of the origin of the branches on the two sides of a node is small, the medullary bundles at first become almost simultaneously curved on both the sides and somewhat horse-shoe shaped before they give rise to the branch traces (Figs. 4C, 5D).

However, in all the nodes, irrespective of the level of insertion of the two branches, the xylem of medullary bundles sooner or later tends to surround the phloem on three sides and the bundles appear like two opposite arcs (or even incomplete rings) whose convex middle points are directed towards the centre of the stem (Figs. 4C, 5D, 8B). In those stems where the branches of the two sides arise at considerably different levels the ends of the two medullary bundles which are on the same side as the higher branch are the first to become curved and the opposite ends develop the curvature later (Fig. 4D, E).

The vascular elements at the two ends of the branch traces form obliquely travelling connections with bundles of the " middle ring " which lie just outside and opposite to the medullary bundles. The curved ends of the medullary bundles towards the higher branch form connections with the leaf supply directly and on the other side indirectly, i.e. they are themselves connected with the branch traces which in turn are connected with the leaf supply (Figs. 2F, 4E, 5C). But in other nodes where the vertical distance between the two branches is small, the medullary bundles become directly connected with the leaf trace elements on both sides.

At a higher level one or both the curved ends of each medullary bundle give rise

TABLE 1

(*Asymmetry of leaves on two sides of midrib*)

Specimen No.	Leaf pair No.*	Larger leaf							Smaller leaf						
		Total leaf length in cm	Width of leaf on either side of midrib in cm						Total leaf length in cm	Width of leaf on either side of midrib in cm					
			At $\frac{1}{4}$th length from apex		In middle		At $\frac{3}{4}$th length from apex			At $\frac{1}{4}$th length from apex		In middle		At $\frac{3}{4}$th length from apex	
			Broader side	Narrower side	Broader side	Narrower side	Broader side	Narrower side		Broader side	Narrower side	Broader side	Narrower side	Broader side	Narrower side
I	1	0·5	0·15	0·15	0·15	0·10	0·12	0·1	0·3	0·09	0·02	0·10	0·09	0·10	0·09
	2	1·2	0·40	0·40	5·20	5·00	0·40	3·5	1·2	0·20	0·20	0·30	0·30	2·50	2·50
	3	1·7	1·70	1·00	1·40	1·00	1·30	1·2	1·4	0·70	0·60	0·90	0·70	0·80	0·70
	4	3·6	1·40	1·30	1·95	1·40	1·80	1·6	2·0	0·90	0·85	1·20	1·10	1·10	1·10
II	1	0·5	0·15	0·15	0·19	0·13	0·10	0·1	0·3	0·90	0·50	0·10	0·08	0·10	0·10
	2	1·1	0·65	0·55	0·70	0·70	0·60	0·5	0·6	0·35	0·30	0·35	0·35	0·29	0·24
	3	2·8	1·30	1·00	1·60	1·20	1·30	1·2	1·4	0·95	0·80	1·00	0·90	0·80	0·70
	4	3·6	1·50	1·50	1·89	1·82	1·70	1·6	2·3	1·40	1·35	1·50	1·40	1·20	1·10
III	1	3·0	1·20	1·15	1·40	1·20	1·30	0·9	1·4	0·75	0·75	0·90	0·80	0·80	0·65
	2	2·9	1·30	1·20	1·60	1·60	1·60	1·5	1·8	1·00	0·95	1·30	1·00	1·20	0·60
	3	3·5	1·60	1·50	1·90	1·70	1·80	1·7	1·6	1·10	1·00	1·20	1·10	1·10	1·00
	4	2·8	1·30	1·20	1·55	1·45	1·40	1·4	1·4	0·75	0·65	0·89	0·80	0·80	0·70

*Beginning with the pair of leaves in the node which is clearly separated from the apical bud by an internode.

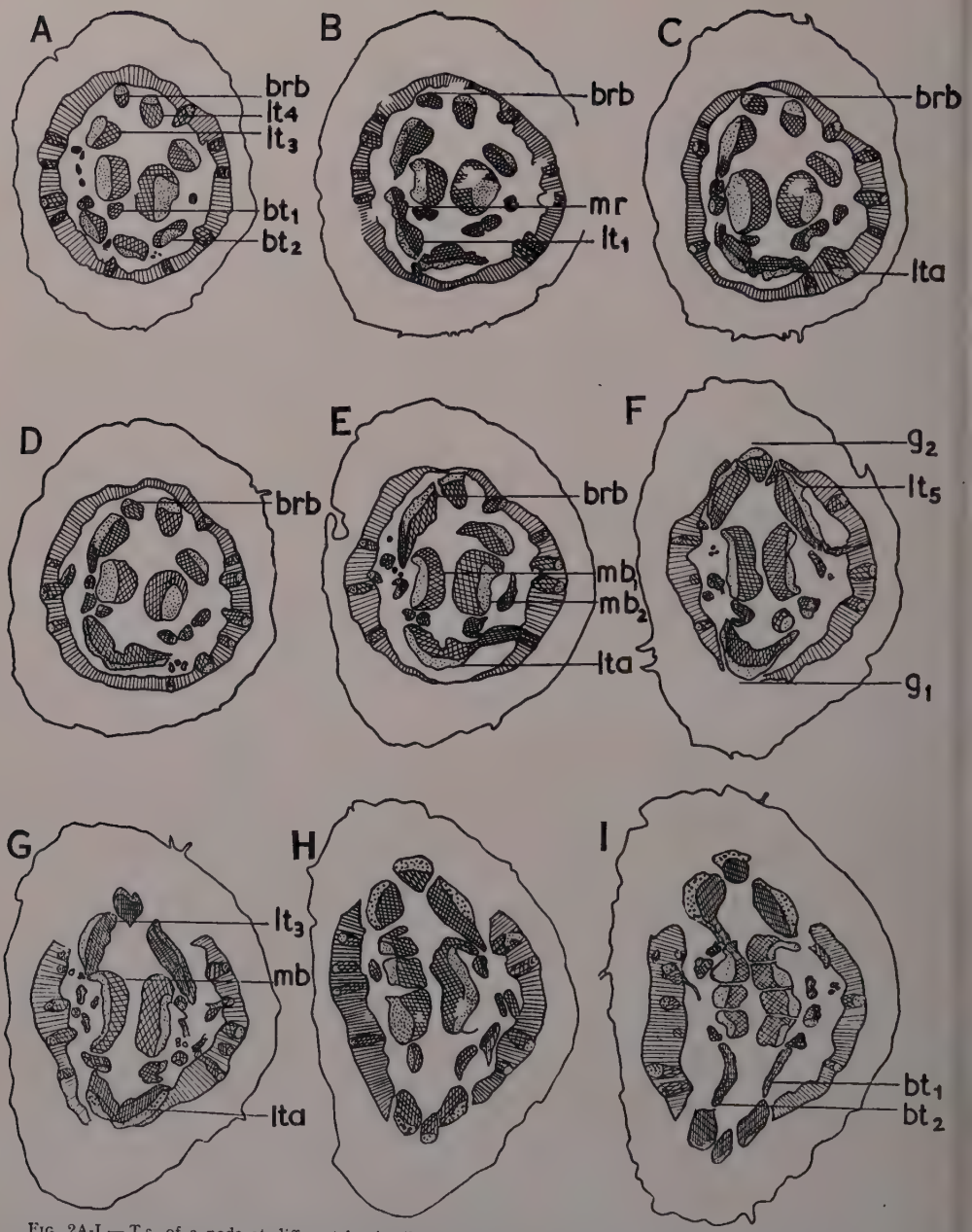

FIG. 2A-I — T.s. of a node at different levels. (*brb*, belated ring bundle; *bt*, branch trace; *g*, gap; *lt*, leaf trace; *lta*, leaf trace arch; *mb*, medullary bundle; *mr*, middle ring bundle.) All, × 25. A. Section showing configuration of bundles just above the lower boundary of node. Two branch traces can be recognized only on one side of the medullary bundles. One of them (bt_2) shows two xylem poles (for details *see* Fig. 5A and its drawing 9A). A bundle from the belated ring (*brb*) is seen deviating towards the centre between two bundles of the "middle ring" which will form the leaf supply. B. Section showing a leaf trace (lt_1) connected with some of the bundles of the "middle ring". C. Section showing the leaf traces of one side fused to form an arc. On one side of the arc a connection has been formed with the belated ring. A trace of the opposite leaf is seen connected with a bundle of the "middle ring". D. Section showing the bipolar branch trace, seen in A-C, divided into two. The belated ring bundle seen in A has now completely separated from that ring and is lying in between two leaf traces. E. Section showing the bundle (*brb*) in D now completely fused with the leaf trace on its left side. The leaf trace arc has once again established a connection with the belated ring. The two sides of the belated ring opposite to the leaf traces have become thinner. The medullary bundles have enlarged. F. Section showing two gaps in belated ring. A leaf trace is seen connected with the belated ring. The curved xylem of the medullary bundles has somewhat straightened out. G. Section showing a medullary bundle connected with a leaf trace (lt_3). The medullary bundles have not yet split, a V-shaped strand (*lta*) has been formed by the fusion of two traces. H. Section showing one of the medullary bundles split into three. I. Section showing the other medullary strand also divided into small unequal bundles. Three leaf traces are seen on the upper and lower sides. On the lower side are also seen two obliquely travelling branch traces.

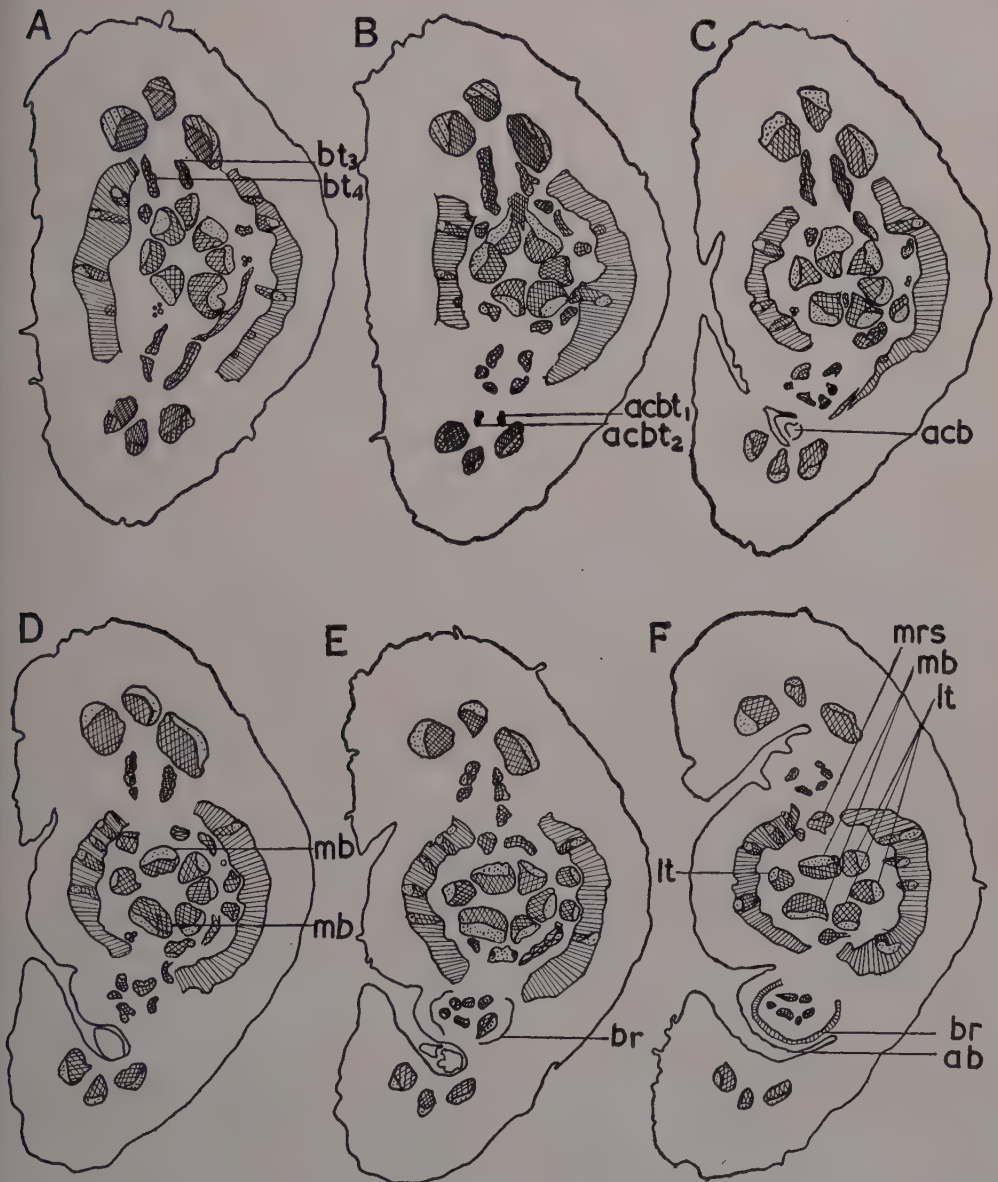

FIG. 3A-F — T.s. of a node at different levels (in the same series as Fig. 2, A-I). (*ab*, axillary branch; *acb*, accessory branch; *acbt*, accessory branch trace; *br*, belated ring; *bt*, branch trace; *lt*, leaf trace; *mb*, medullary bundle; *mrs*, middle ring strands.) All, × 25. A. Main axis showing ring of vascular strands in the centre. Two more branch traces have also become differentiated. B. A pair of bundles of the ring opposite the upper leaf have fused. The axillary branch strands are arranged in a single ring. The accessory branch traces have also differentiated. C. The accessory branch has started, separating. D. Two bundles (*mb*) in the main axis have become enlarged; others have deflected outwards. So far no belated ring is seen around the vascular strands of the axillary branch. E. A belated ring has started differentiating in the axillary branch with gaps towards parent axis and accessory branch. F. An incomplete belated ring has become differentiated in the axillary branch. The gaps in the belated ring of the parent axis and the axillary branch face each other, the parent axis once again tends to assume the internodal arrangement of bundles, a middle ring strand (*mrs*) is filling one of the gaps in the belated ring, the leaf trace strands lie on the two sides of the medullary bundles.

to small vertical vascular strands (on both sides in nodes where the vertical distance between the two branches is small and only on the side of the upper branch where the vertical distance is relatively large). In some cases phloem ends of such strands may remain fused for some distance and their transverse sections show a single central phloem surrounded on its sides by groups of xylem cells (Figs. 5B, 9B).

Higher up in the node the two medullary bundles broaden out and once again become almost flat. In transverse sections they now look like two somewhat wide parallel strips of vascular tissue (Fig. 2F). These strips split up into a number of small unequal vascular strands. Such strands are separated merely by thin plates of parenchyma (Fig. 2H, I). While the strands are still lying closely grouped together, the median strands produced by each medullary bundle shift towards the periphery, at successively higher levels, so that they now seem to be arranged in two arcs with their concavities towards the centre (Figs. 2I, 3A). The level at which the medullary bundles split into these strands may vary from node to node. In some of the nodes the medullary bundles have already divided at as low a level as the region where the two ends of the medullary bundles start appearing curved (Figs. 4C, 5D). In others they do not split till they have given rise to the branch traces (Fig. 2A-G). The number of strands formed from each of the two parallel strips of medullary vascular tissue may vary, but generally one of them splits into three strands and the other into four (Fig. 2I).

At a higher level the bundles of the two arcs formed by the splitting medullary bundles become arranged in a ring (Fig. 3A). In this ring a pair of bundles on either side of the node which lie in the direction of the two leaves (i.e. at right angles to the medullary bundles of the internode below) become broader as a result of fusion with each other or with strands from other rings (Figs. 4F, 11A). These form the medullary bundles of the next internode. Two bundles of each pair of such strands either approach each other and fuse directly or are bridged by a strand coming very obliquely from the next outer ring. In some cases, at about the same level the free curved ends of one or both of these fused bundles separate simultaneously or successively. Occasionally smaller bundles of the ring formed by the split medullary bundles may also fuse or divide. These diversities in the fusion and separation of the various bundles are thus responsible for varying number of the bundles seen at a higher level in the node.

Sometimes one of the two medullary bundles is formed by the direct fusion of a pair of strands as mentioned above but the other one is formed in a somewhat different manner. The pair of bundles on its side may at first appear to be bridged by a strand coming obliquely from the " middle ring " but the identity of each individual strand in the pair is never lost. As a result only one of the two bundles fuses with a third strand and ultimately turns into the second medullary bundle. The other bundle of the pair once again becomes separated from it by a wide sheet of parenchyma. As a matter of fact at this stage the two large so called medullary bundles and other smaller bundles are still arranged in a single initial ring (Fig. 3F), and in some sections this is surrounded only by the belated ring, there being no " middle ring " bundles at such levels (Figs. 4B, 7C). The smaller strands of the ring travel obliquely outwards so that at a higher level these and some other bundles which arise from the outer ends of the fused pairs of bundles appear to form the " middle ring " of loosely arranged bundles while the two large bundles pursue a straight vertical course and seemingly become medullary in the next internode (Figs. 3, 4A, B). In some of the nodes the small bundles of the " middle ring " become the connecting bundles between the initial ring and the belated ring (Fig. 3F).

After attaining this arrangement no appreciable change takes place in the topography of the bundles and the general organization of the stem. Figure 3F is now actually in the next internode. A change in the arrangement and form of bundles will hereafter reappear only when we approach the next node and again and

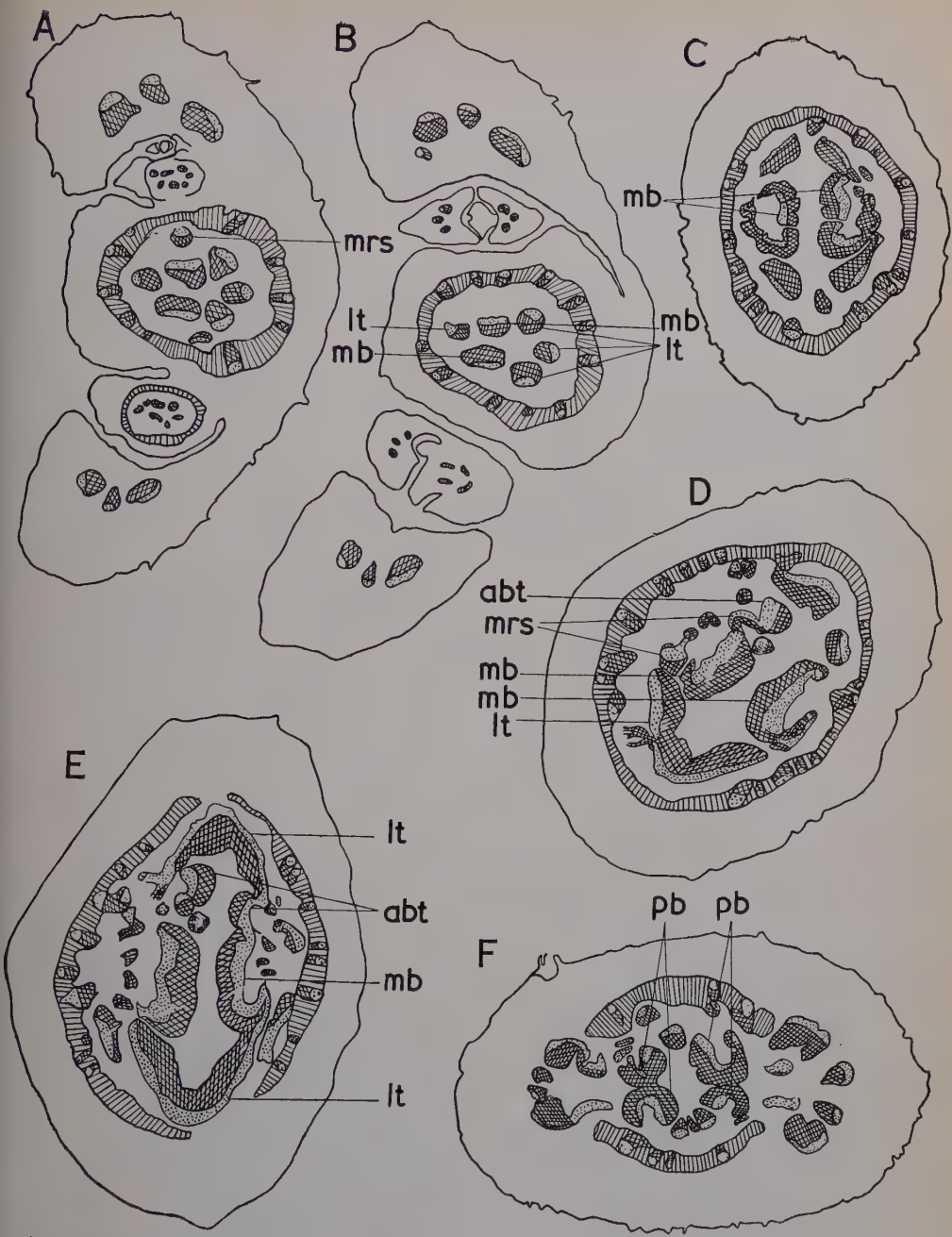

FIG. 4A-E — T.s. at different levels of nodes (A, B are in the same series as Figs. 2A-I, 3A-F). (*abt*, axillary branch trace; *lt*, leaf trace; *mb*, medullary bundle; *mrs*, middle ring strands; *pb*, fusion of a pair of bundles). All, × 25. A. The gaps in the belated ring of the main axis and an axillary branch have become closed. The main axis shows two larger medullary bundles and other smaller bundles of the "middle ring" which form the supply of leaves of the following node and one of them (*mrs*) gets deflected into the belated ring. The two petioles on either side of the main axis show three bundles each. B. The petiole of the smaller leaf has completely separated, the opposite petiole of the larger leaf has four vascular bundles. The medullary bundles of the main axis are lying parallel to the two leaves but at right angles to the medullary bundles of the previous internode; leaf traces are seen on either side of the medullary bundles (*cf.* Fig. 2A). C. T.s. of a node (same as photograph Fig. 5D), showing medullary bundles with curved ends. D, E. T.s. of a node showing an almost dumb bell-shaped complete ring of bundles which are at places connected with bundles of the belated ring, the curved ends of medullary bundles show connections with leaf traces, axillary branch traces and middle ring strands (*see* also Fig. 5C). F. T.s. of a node showing fusion of two pairs of bundles opposite the two leaves. The number of petiolar strands varies, three on one side and four on the other (*see* also Fig. 11A).

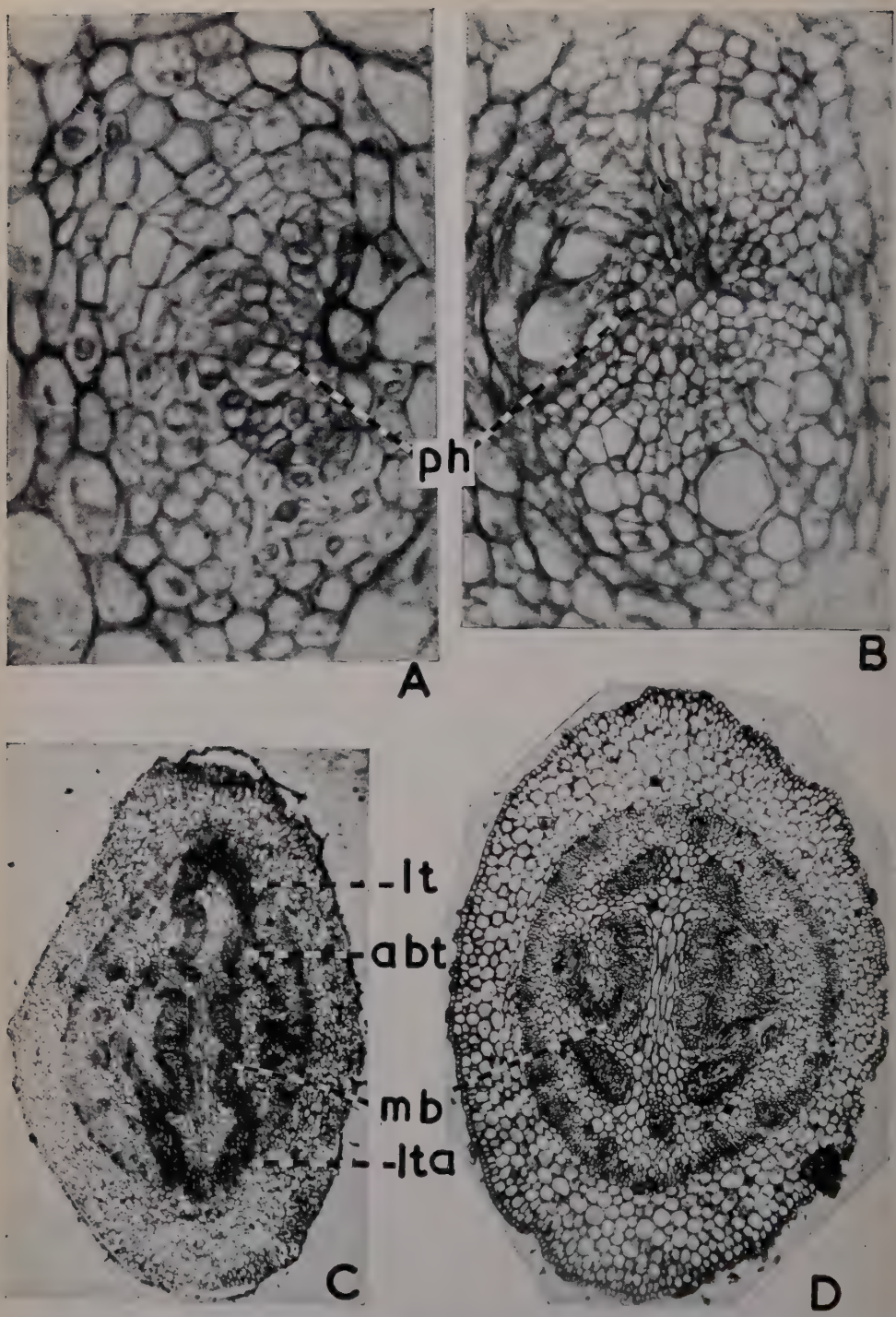

FIG. 5A-D — (*abt*, axillary branch trace; *lt*, leaf trace; *mb*, medullary bundle; *ph*, phloem). A. A bipolar vascular bundle (branch trace) showing xylem on two sides of phloem. × 700. B. A transversely cut vascular strand from the nodal region showing xylem all around phloem. × 287. C. T.s. through a node showing an almost complete dumb bell-shaped ring of vascular bundles formed by the fusion of leaf traces, axillary branch traces and medullary bundles. × 28. (The ring is more complete in the section figured in 4E). D. T.s. through a node to show two curved medullary bundles and their connections with the strands of the "middle ring" which are lying opposite to them. × 42.

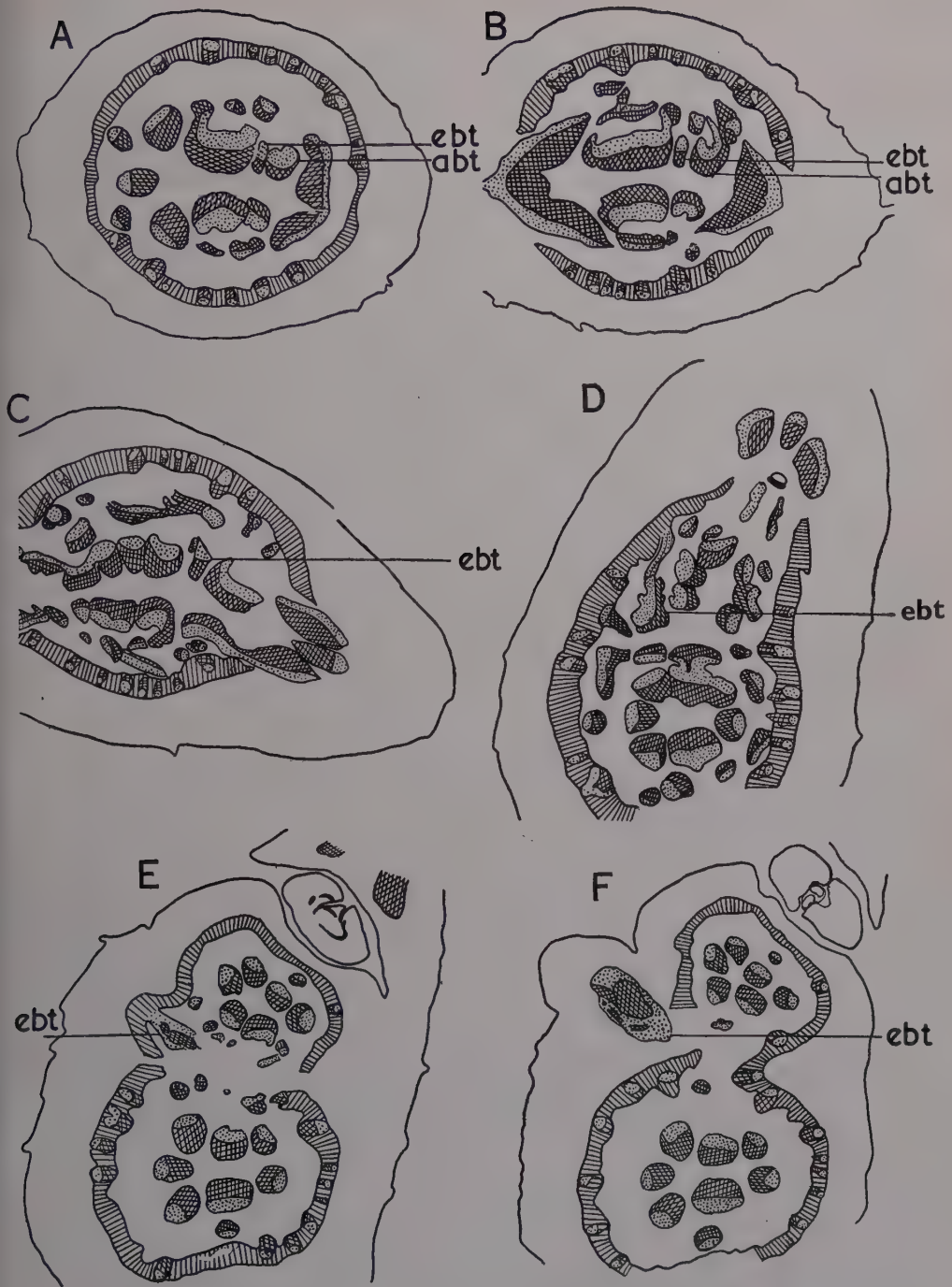

FIG. 6A-F — A series of transverse sections of a node at different levels showing the origin of the vascular supply of an extra-axillary branch. (*abt*, axillary branch trace; *ebt*, extra-axillary branch trace). All, × 25. A. The axillary branch trace has given rise to a small bundle (*ebt*) which is still lying in close contact with it (*see* also Fig. 11C, E). B. The extra-axillary branch trace is now lying closer to the medullary bundle. C. The trace (*ebt*) in B has become joined on the phloem side of a bundle of the "middle ring". The xylem strands are separate. D. The trace (*ebt*) in C has become larger in size and is connected obliquely with the belated ring. E. The belated ring has formed a small bulge and it is connected with the bundle of the extra-axillary branch. F. The trace *ebt* is seen travelling obliquely through the cortex leaving a gap in the belated ring.

again the same series of changes will take place in each node. Thus, it is clearly shown that in each successive internode the medullary bundles come to lie at right angles to those of the previous one (cf. Figs. 2A, 4B) and that the branch traces are mainly derived from the nodal medullary bundles. The leaf traces too are connected with the nodal medullary bundles but these are only weak connections and the main traces of leaves at a particular node are actually formed in the previous node.

The Origin of the Leaf Traces — As already described there are present, on either side of the medullary bundles, usually two or three, occasionally only one and rarely more than three bundles, which are of a medium size (they are somewhat smaller than the two medullary bundles but larger than some other bundles of the " middle ring "). Such medium sized bundles which are included in the " middle ring " ultimately form the leaf supply (Fig. 13A).

Towards the node the bundles forming the leaf supply become wider and approach each other. Simultaneously some of the bundles of the belated ring lying opposite to these travel obliquely inwards and bridge the gap between these strands. In the supply of other leaves, one or more bundles of the belated ring may bend obliquely and join some of the individual bundles which are to enter the leaves (Fig. 2A-E).

In a large number of nodes the various strands forming the leaf supply become fused at the margins and give rise to a " C " shaped strand (Fig. 2C, G). However, in quite a few nodes the fusion is never complete although the bundles lie close to each other (in some they may even remain rather far apart), and separate strands like these may later divide in the region of the leaf base. The variations in the course of the leaf trace may occur even in the supply of two opposite leaves; the traces of one may remain separate while those of the other may fuse before they enter the leaf base.

The margins of the " C " shaped transversely cut leaf traces or the bundles at the ends of their " C " shaped arc become connected at different levels, with obliquely travelling strands of the belated ring (Figs. 2C-E, 4D, 10A). They may also join with other small bundles of the " middle ring " (Fig. 2B). As already described (on page 386) the ends of the leaf trace bundles may become connected at higher levels either with the branch traces or with the medullary bundles. Whether in a fused condition or separate, these leaf traces always travel obliquely through the cortex (Fig. 10A). Sooner or later, the single " C " shaped strand formed by the fusion of the leaf traces breaks up into three somewhat unequal strands which later redivide and form strands of different sizes (Figs. 4A, B, F). Their number varies a great deal, in some there are only three strands for a considerable distance in the petiole, in others there may be as many as seven unequal vascular bundles. Occasionally some of these strands fuse again in the petiole, and sometimes, the number of the strands in the two petioles of the same node and also of different nodes in the same plant, varies, at other times it is the same in both of them. The smaller leaf is the first to separate and the larger leaf separates thereafter (Fig. 4B).

Origin of the Branch Traces — Two axillary branches on either side of a node are never exactly opposite but each individual node may show a considerable variation in the vertical distance between the points of origin of the traces leading into the two branches. In accordance with the difference in the levels of the origin of the two branches, the branch strands, one pair on each side of the medullary bundles, may separate from them at considerably different levels or almost simultaneously.

In the nodes in which the vertical distance between the two branches is appreciable, the strands of the lower branch separate from the medullary bundles at the base of the node (Fig. 2A). The traces of the higher branch separate when the medullary bundles flatten out after the stage when they appear like two arcs in transverse sections (*see* p. 386). On the side of the higher branch two small vascular strands separate from the curved ends of the medullary bundles (one from each bundle) which

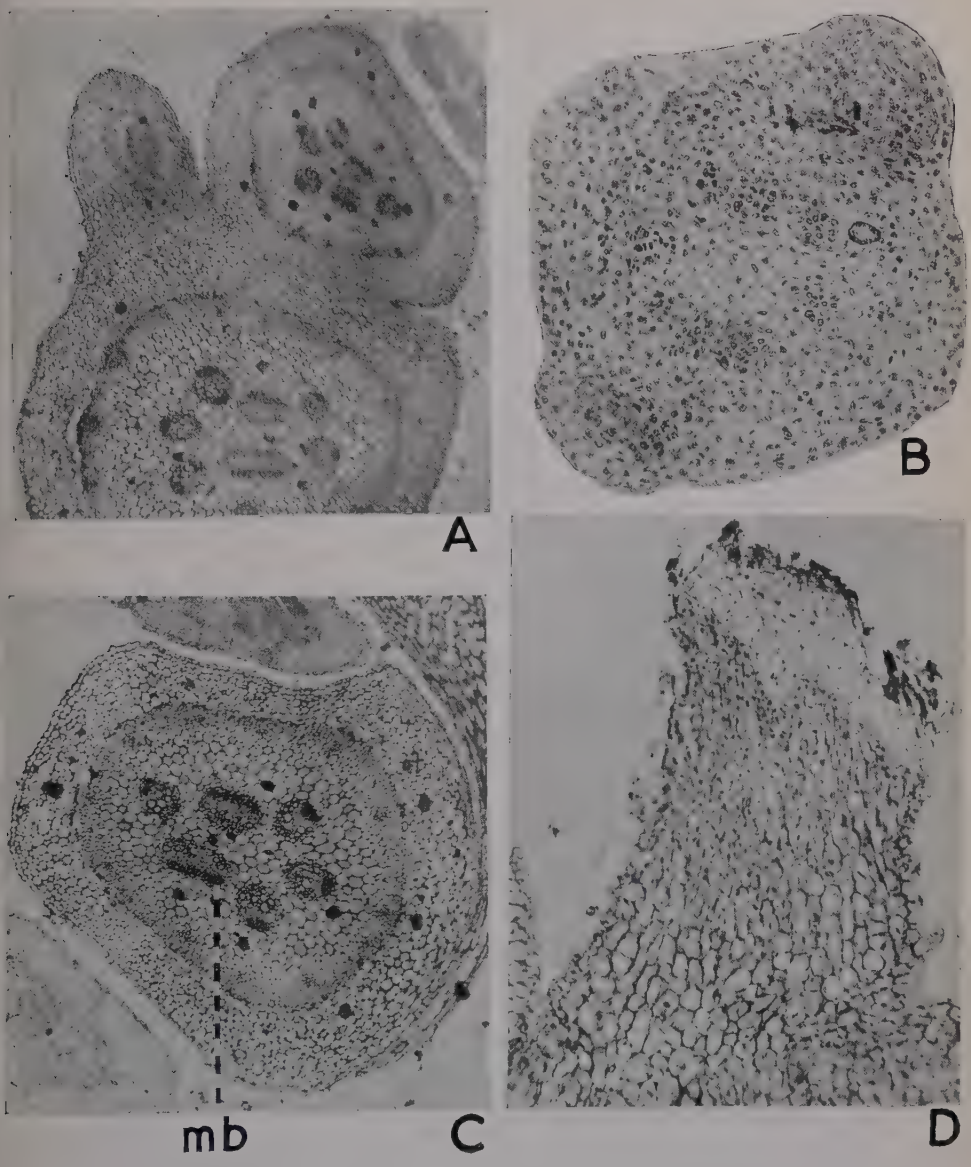

FIG. 7A-D — (*mb*, medullary bundle). A. T.s. of a node through base of an axillary and extra-axillary branch. × 31. B. T.s. of a young axillary branch just near its apex showing a single ring of procambial strands surrounding a well differentiated pith. On two sides of the branch axis are seen the apices of two branchlets. × 182. C. T.s. through the upper region of a node showing single inner ring formed by separate bundles and a belated ring (at this stage no definite "middle ring" can be recognized), two medullary bundles are facing each other (*see* Fig. 4B). × 42. D. Longitudinally cut base of extra-axillary branch (peduncle) showing seriated cork cells of abscission layer. × 114.

during their later course become the traces of the higher axillary branch (Figs. 2I, 3).

When the vertical distance between the two branches is relatively small, the branch traces on both the sides separate almost simultaneously from the curved ends of the medullary bundles at a relatively higher level. In some nodes the traces of the lower axillary branch also show a tendency to become curved like the medullary bundles (Fig. 2F), and they too have their convex sides facing each other. Occasionally such strands show two xylem points on either side of a central phloem strand (Figs. 2A, 5A, 9A). The curved ends of the branch traces become obliquely connected with adjoining small strands of the " middle ring " (Fig. 4D). At a higher level in the node each of the two branch traces get connected with the medullary bundles on one side and with the leaf traces on the other (Figs. 4E, 5C).

Higher up in the nodes young axillary branch traces are seen to lie as two parallel strips of vascular strands (Figs. 2I, 3A). Later, they split and form two bundles towards the subtending leaf, and these form the traces of the accessory branch (Fig. 3A, B). In some older nodes the accessory branch traces may again give rise to two more strands towards the subtending leaf, in the same manner as the traces of the axillary branch produce the first accessory branch traces. These supply a second accessory branch. After giving rise to the traces of the accessory branch, the strands of the axillary branch divide into a number of small bundles in much the same way as the two medullary vascular plates divide to form two parallel rows of bundles in the node. Higher up the vascular strands of the axillary branch become arranged in a single ring (Fig. 3A-D). At a still higher level all of them, except two, travel obliquely outwards to form a " middle ring " of loosely arranged bundles, while the two bundles travelling straight assume what may now be called the medullary position (Fig. 3E, F).

In a young axillary branch the belated ring of bundles develops after the differentiation of the inner rings (Figs. 7B, 12A, B). At the base of a branch the belated ring differentiates still later: after it develops there it ultimately becomes connected with the two sides of the gap in the belated ring of the parent axis. In older nodes rings of vascular bundles and conjunctive tissue are formed outside the first belated ring; these may be connected with, but do not affect the course of the pre-existing vascular bundles.

The Origin of the Extra Axillary Branch Traces — It has already been mentioned in connection with the external morphology of the node that it shows an extra-axillary branch (the inflorescence stalk) in the reproductive phase of the plants (Fig. 1A, C).

Immediately after separation from the medullary bundles, one of the axillary branch traces gives rise to a small bundle on its side towards the adjacent medullary bundle (Figs. 6A, B, 11C, E). For quite a distance this bundle lies in between the medullary bundle and the axillary branch trace without any appreciable change in its form or position. In only one of the nodes studied by us it was ultimately found to travel towards the medullary bundle and then came to lie by its side and finally lost its identity with the medullary bundle (Fig. 8A), but in some other nodes it becomes connected with the adjacent medullary bundle by means of a thin oblique connecting strand and in still others there may be no connection formed in between the medullary bundle and this strand throughout its course.

At a higher level this strand also establishes oblique connections with the bundles of the " middle ring " and is also strengthened by some vascular strands from the belated ring (Fig. 6C-E).

This augmented strand then travels obliquely outwards and in some nodes, passes out into the cortex through the gap in the belated ring which is by now formed for the axillary branch. In other nodes it passes out through a gap of its own. Simultaneously with its deflection towards the periphery, a bulge appears around it in the outline of the cortex, slightly towards one side of the smaller leaf base (Fig. 6E, F). Thereafter the extra-axillary branch trace breaks up into a few strands (Fig. 9C) as the branch base separates from the parent axis.

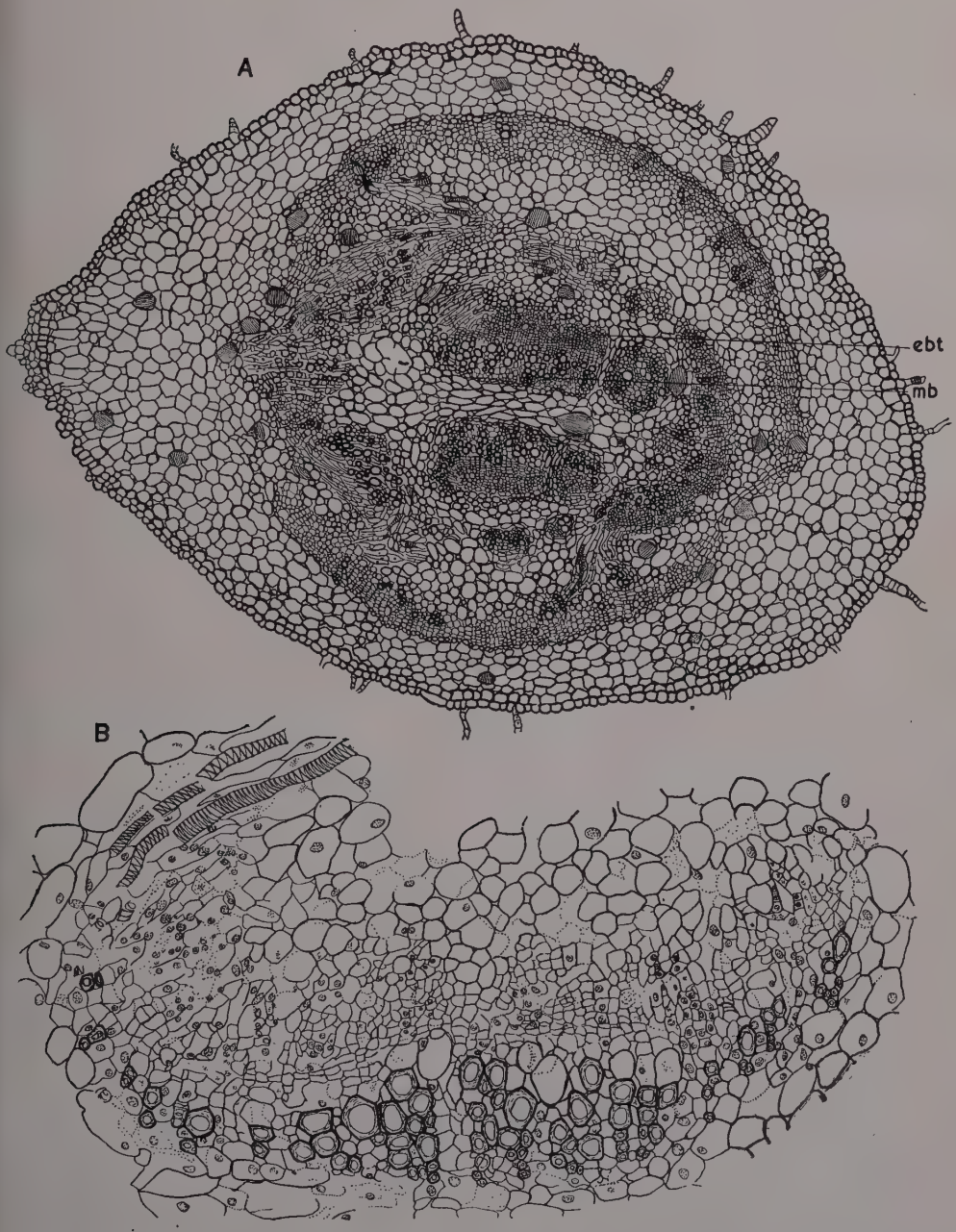

FIG. 8A-B — (*ebt*, extra-axillary branch trace; *mb*, medullary bundle). A. T.s. of a node in the same series as Fig. 6, at a level between sections B and C of Fig. 6, enlarged to show details. The extra axillary branch trace is completely fused with the medullary bundle. × 50. B. A single curved medullary bundle showing xylem on three sides of phloem. × 167.

Course of the Bundles of the "Middle Ring" — As described by Maheshwari (1930) the "middle ring" in the internode consists of a few loosely arranged unequal vascular strands. Of these usually two or three strands lying towards the two lateral flanks of the medullary bundles become the leaf traces in their later course (Figs. 2A-I, 5D). Other smaller bundles which lie in the parenchyma, between the phloem of the medullary bundles and the belated ring, may take up an oblique course as soon as they enter a node and become connected with the curved ends of the medullary bundles, the main leaf traces or the branch traces. Some other smaller bundles come to the "middle ring" from the belated ring by taking a slightly centripetal course or enter the belated ring by bending outwards in the centrifugal direction (Figs. 2A-E, 3F, 4A). At different levels in the region of the node, the obliquely travelling strands of the "middle ring" become connected with the fusing pairs of bundles which will ultimately form the medullary bundles of the next internode. From the upper sides of such oblique connections with the pairs of bundles arise a number of small vertical strands (Fig. 11A), most of which run between the medullary bundles of the next internode and the belated ring but usually a few of them travel centrifugally to enter the belated ring. However, in some other nodes it was observed that all such smaller strands gradually travel outwards and enter the belated ring — thus filling its two gaps which are formed in the region of the node (Fig. 3F). In such nodes, therefore, no bundles remain in the "middle ring" except for the leaf traces on either side of the medullary bundles (Figs. 4B, 7C).

Course of the Bundles of the Belated Ring — In the internode the belated ring formed by vascular bundles and intervening conjunctive tissue forms a continuous cylinder around the various inner strands (Fig. 13A). However, on entering the node the bundles of this ring join with small bundles of the "middle ring" chiefly in the interpetiolar regions (Fig. 4E; *see* also the numerous anastomoses between bundles in Figs. 11D, 12C). The strands of the belated ring also strengthen then the leaf traces by means of connections or they themselves travel obliquely inwards and either form bridges between two of the leaf trace strands or join an individual strand on one side (*see* page 394 and Figs. 2C, E, 4D).

Higher up in the node just opposite the leaf traces there are formed two gaps in the belated ring so that it appears divided into two semilumar halves (Figs. 2F-I, 3A-F). After the leaves and the branch strands have departed the gap is once again filled up by differentiation of a complementary arc of belated ring above the gap into which sometimes some of the bundles of the "middle ring" get deflected (Fig. 4A, B).

Discussion

The node of *Boerhaavia diffusa* presents a fairly complicated anatomy, perhaps because of the occurrence of at least three apparent rings of vascular bundles. However, to begin with there is a single ring of procambial strands in a young axis (Figs. 7B, 12A, B) and these mature into an inner ring of bundles but as pointed out on page 390 some of it's bundles become deflected outwards in the node below forming the so called "middle ring" of leaf traces and other minor strands in the internode. The bundles of the belated ring originate outside the first ring. In the nodes the medullary bundles form wide plates which later split up into a number of strands from which the medullary bundles of the next internode are differentiated at right angles to those of the previous one.

The traces of the leaves of a succeeding node are already differentiated in the upper part of the previous node. The traces of the branches of a node are, however, formed in the same node. Once formed the foliar strands of a node travel through the whole of the previous internode as the bundles of the "middle ring". In *Arjona, Cervantesia, Omphacomeria* and *Exocarpus* also, three leaf traces travel all through an internode in a similar manner (Swamy, 1949). When these bundles enter the nodal region where they depart to a leaf, they take up an oblique course and pass out separately

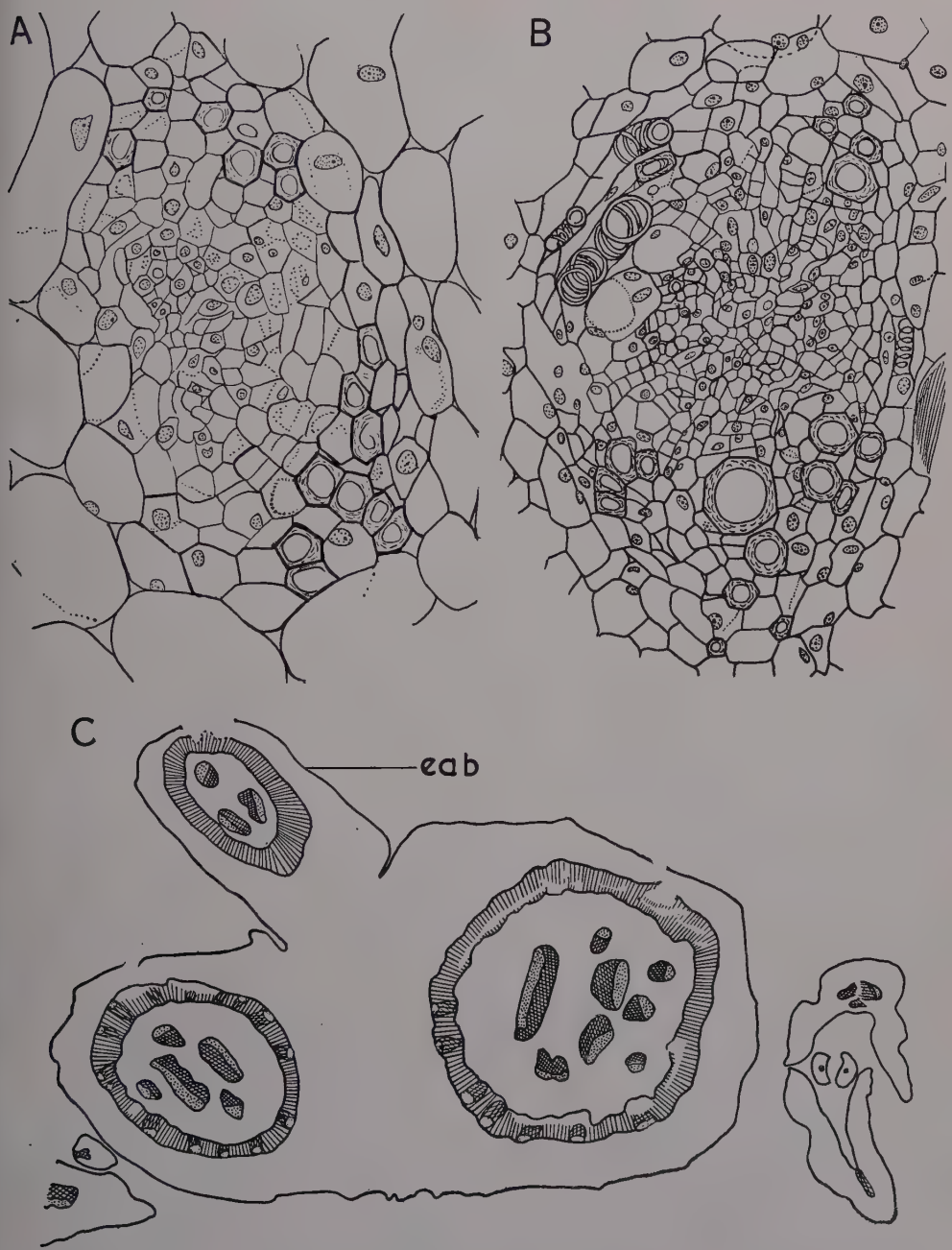

FIG. 9A-C — (*eab*, extra-axillary branch). A. Branch trace with bipolar xylem in Fig. 2A and 5A — further magnified to show xylem on two sides of phloem. × 500. B. A transversely cut strand from the nodal region showing xylem elements all around phloem. × 250 (*see* also Fig. 5B). C. T.s. of a node showing three vascular strands in the extra-axillary branch surrounded by the belated ring. × 25.

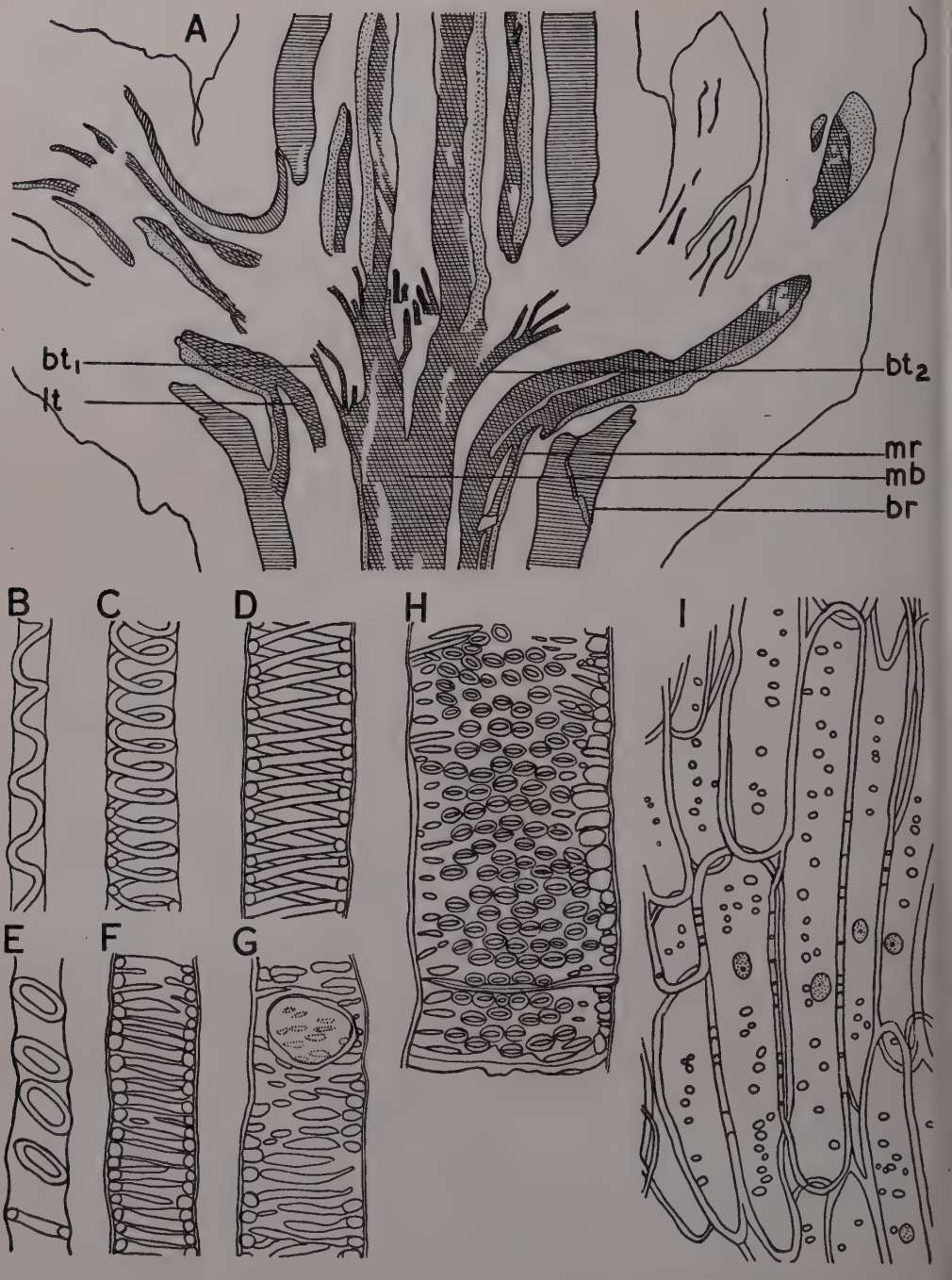

FIG. 10A-I — (*br*, belated ring; *bt*, branch trace; *lt*, leaf trace; *mb*, medullary bundle; *mr*, middle ring). A. L.s. of a node showing longitudinally cut belated ring, " middle ring " and one of the medullary bundles which has divided into two main strands and a few smaller ones in the upper part of the node. On the two sides branch traces are seen arising from the medullary bundle and on the left side is seen a clear connection between the leaf trace and the belated ring. The leaf on the right bears two buds in its axil. × 25. B-H. Xylem elements showing various types of thickenings. × 500. I. L.s. conjunctive tissue cells showing scattered simple pits and nuclei. × 500.

or after fusion. The foliar supply and nodal anatomy of *Bougainvillaea spectabilis* and *Mirabilis jalapa* show a good deal of resemblance with *Boerhaavia diffusa*. In these plants also three bundles of the central ring were seen to form the leaf traces after getting connected with other outer bundles at different levels. In *M. jalapa* the leaf traces completely fuse with each other before entering the leaf base, but in *Bougainvillaea spectabilis* the three leaf traces remain separate. *Boerhaavia diffusa* presents a somewhat intermediate condition, here the traces of some leaves may completely fuse and redivide before entering the leaf base while in others they pass out without complete fusion.

The departure of the leaf traces results in the formation of a single leaf gap in the belated and the middle rings of *B. diffusa*. This condition conforms with Sinnott & Bailey's (1914) observation that the node in Nyctaginaceae is typically unilacunar. However an unusual feature of the node in *B. diffusa* is the variable number of the strands supplying a leaf (1-3); in most other angiosperms their number is constant. Even their course and the manner in which they enter a leaf base are variable. In addition the leaf traces often get strengthened by a variable number of smaller bundles from the middle and belated rings.

The two leaves of a node in *B. diffusa* are generally described as opposite and decussate but strictly speaking they are not always so. A study of sections through a node clearly shows that the point of attachment of the smaller leaf is slightly lower than the larger one and mostly in older nodes the angle between the petioles of the two opposite leaves is slightly greater than 180° on one side than on the other (*see* Fig. 4B). *M. jalapa* presents a similar case and its leaves too arise at slightly different levels, but externally they appear to be opposite. It may be pointed out that in some other plants also the leaves are apparently at the same level but the internal structure shows that they are actually inserted at somewhat different levels, e.g. in *Peperomia fenzlei*, *Peperomia prostrata* and *Peperomia comarapana* (Murty, 1960); the angles between the two opposite leaves in these species of *Peperomia* are, however, not described.

As pointed out on page 384 older nodes of *B. diffusa* frequently show accessory branches in the axils of leaves. A similar condition has been reported by Franks (as quoted by De Bary, 1884) in *Rubus* and by Garrison (1955) in *Juglans cinerea*. In both these forms and in *B. diffusa* two accessory branch traces arise from the distal ends of the two axillary branch bundles. Nevertheless, unlike *Rubus* and *Juglans* the outer ends of the accessory branch traces never meet in *B. diffusa*. The origin of the extra-axillary branch trace is quite in consonance with its interpetiolar position.

In the nodes as well as in the internodes, sections of stems of *B. diffusa* may occasionally seem to show a somewhat plate-like arrangement of bundles (Fig. 13A) instead of regular rings; most of the bundles appearing arranged in line with the medullary strands or in plates parallel to them. This may give rise to the suspicion that this apparently plated arrangement of bundles may be the result of the prostrate habit of this species of *Boerhaavia*. Our study of the nodes of the plant, however, clearly points out that the peculiar arrangement of the bundles in its stems follows a decussating sequence like that of its leaves but in successive nodes the decussate leaves become parallel to the ground due to a twisting of their petioles. In order to confirm this conclusion we grew some stems erect on supports and the portions of such stems which are formed erect did not show any abnormality in their anatomy.

Summary

External features of *Boerhaavia diffusa* L. usually show one or more accessory buds in the axil of each leaf, and an extra axillary branch, only on the side of the small leaf. The nodal anatomy has been investigated in some detail. It was found that as the two so called medullary bundles enter the node, their xylem tends to surround the phloem on three sides. Curved ends of these strands become obliquely connected with bundles of the other

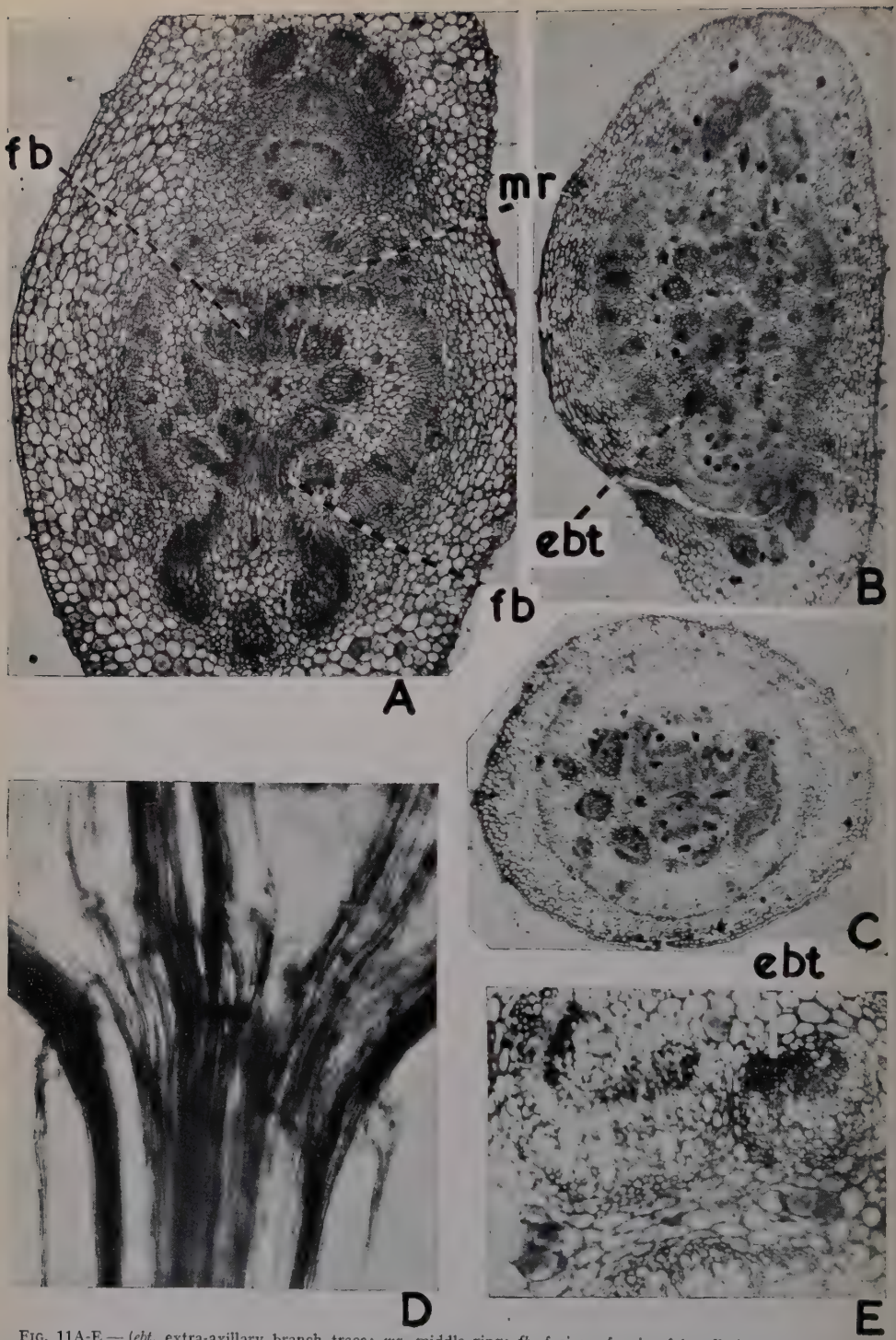

FIG. 11A-E — (*ebt*, extra-axillary branch trace; *mr*, middle ring; *fb*, fusion of pair of bundles). A. T.s. through a node to show the fusion of pairs of bundles of the inner ring lying opposite to the leaves, and the formation of the smaller bundles of the middle ring. × 42. B. T.s. of a node showing the extra-axillary branch trace cut at a higher level in the node. × 28. C. T.s. through a node showing three leaf traces on one side and a single arc of bundles formed by the fusion of leaf traces on the other side, an extra-axillary branch trace is seen lying close to the axillary branch trace. × 28. D. Cleared whole mount of a stem showing xylem strands in a node. × 42. E. A part of C magnified to show the extra-axillary branch trace. × 87.

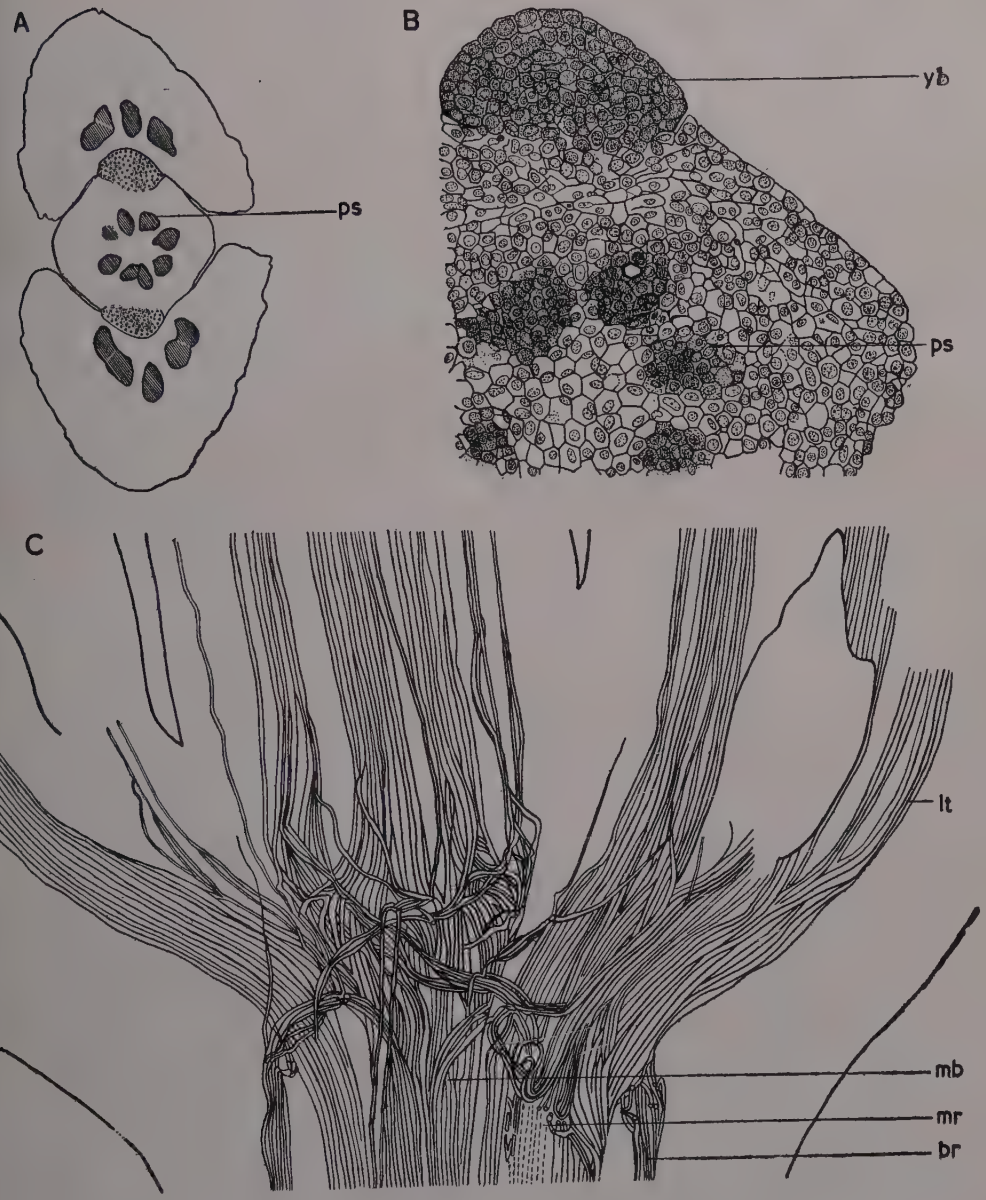

FIG. 12A-C — (*br*, belated ring; *lt*, leaf trace; *mb*, medullary bundle; *mr*, middle ring; *ps*, procambial strand; *yb*, young branch). A. T.s. of a young branch with two leaves. The axis shows a ring of procambial strands and two primordia of axillary branches. × 70. B. A sector of A more magnified to show details (*see* also Fig. 7B). × 280. C. Xylem strands of a node as seen in a whole mount transparency. (*see* also Fig. 11D). × 40.

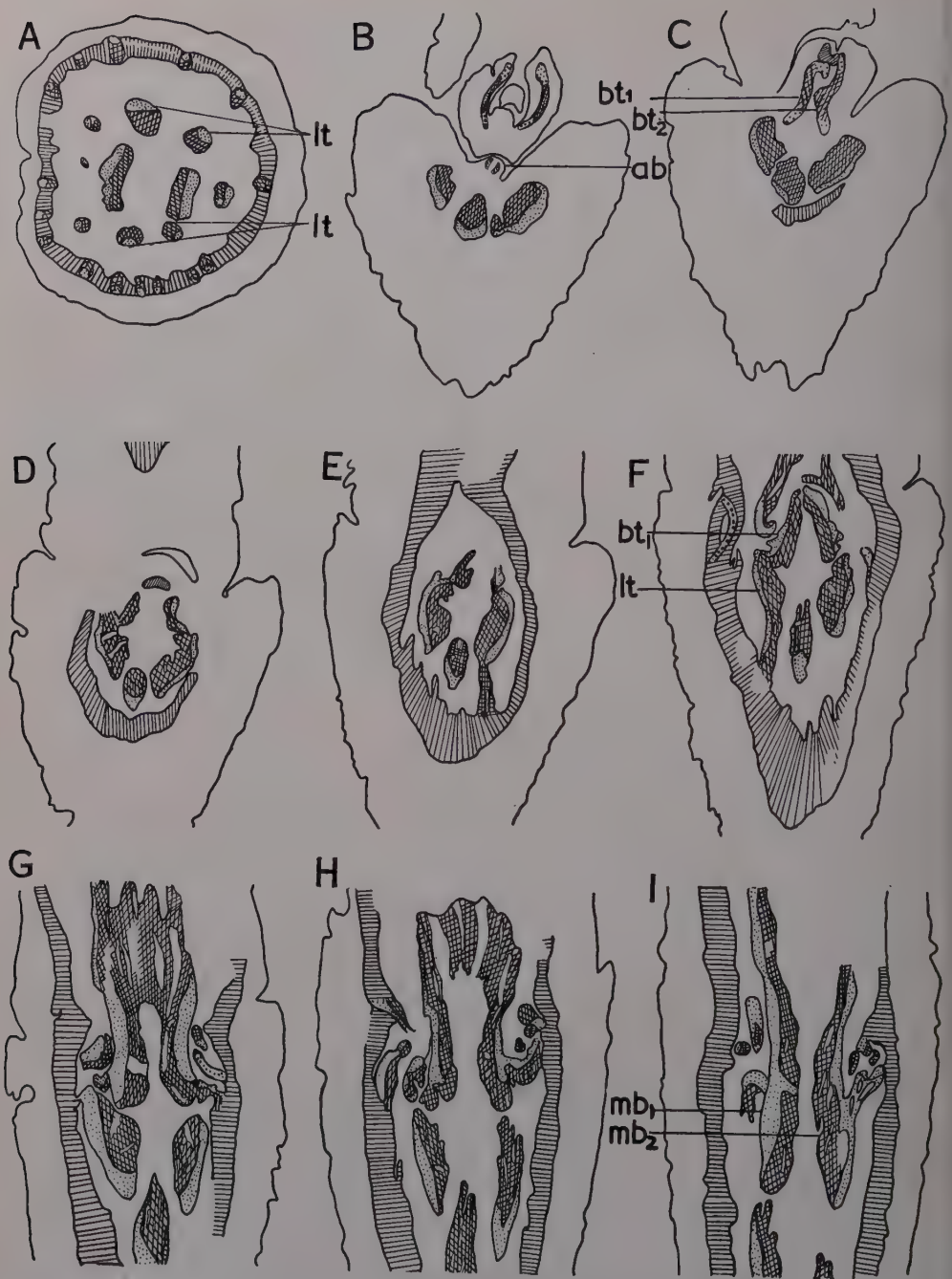

FIG. 13A-I — (*ab*, accessory branch; *bt*, branch trace; *lt*, leaf trace; *mb*, medullary bundle). A. T.s. of a young internode showing arrangement of vascular bundles, the middle ring bundles lying on two sides of the medullary strands are the leaf traces. B-I. A series of longitudinal sections of a node cut at right angles to the plane of the leaves beginning with the tangential section B through intervening levels to an almost median section I. B. Section of petiole with three strands and two axillary branches, the accessory branch is seen between the axillary branch and the petiole. C. Section showing basal part of petiole connected to axillary branch. An arc like belated strand is seen on the abaxial side of the petiolar bundles. The axillary bud shows two almost longitudinally cut, branch traces. D. Section showing a leaf trace connected with the belated ring. F. Section showing another leaf trace in connection with the belated ring below and with the branch trace above. G, H. Sections showing three leaf traces below and longitudinally travelling medullary strands above. Some bundles in the node which travel horizontally are cut transversely. I. Median section showing medullary bundles connected with the belated ring and a few strands of the " middle ring ". All, × 25.

rings at different levels. The two medullary bundles then split up into small unequal strands which become arranged in a ring. Mostly a pair of strands lying on either side towards the leaves fuse to form the medullary bundles of the next internode and other bundles form the "middle ring". The medullary bundles of each internode lie at right angles to those of the previous one.

Leaf supply is mainly formed by the bundles of the "middle ring", usually two or three of them lying on two sides of the medullary bundles become the main strands of the leaf. The leaf traces may fuse or may remain separate while passing into the leaf base. The number of bundles in the petioles varies in different regions.

The branch traces arise mainly from the medullary bundles. The axillary branch traces give rise to two small abaxial strands which form the supply of the accessory branch. Usually one of the branch traces lying on the side of the small leaf cuts off a small strand towards the medullary bundle, which in its later course forms the supply to the extra-axillary branch.

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MORPHOLOGY AND CLASSIFICATION OF THE TREE FERNS

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Most large tree ferns belong either to the genus *Cyathea* (including *Hemitelia* and *Alsophila*) or to *Dicksonia*. In the days when the position and structure of the sorus were the over-riding criteria in classification, these two genera were placed in distinct tribes, because the sori in *Dicksonia* are marginal (at the ends of veins) and those of *Cyathea* are on the lower surface of veins. In *Dicksonia* each sorus is protected by a more or less modified small lobe of the leaflet (the outer indusium) and also by an inner indusium of about the same size and not greatly different in substance. In *Cyathea* the sori are protected by indusia of a variety of forms, or have no indusia.

When attention began to be given to dermal appendages as of taxonomic significance, it was noted that the young parts of *Cyathea* are protected by scales (with some hairs also), those of *Dicksonia* only by hairs.

Associated with *Cyathea*, because of superficial sori (in both cases without indusia) were the two monotypic and very dissimilar tropical American genera *Lophosoria* and *Metaxya*; but both these genera have hairs, not scales, as a protection for their young parts.

Associated with *Dicksonia*, and often included in it as subgenera, were: *Cibotium*, very distinct in its firm pale indusia; *Culcita*, distinct in the asymmetric shape of leaflets and in the form of the upper surface of its axes, but for a long time not clearly characterized; *Cystodium*, similar to *Dicksonia* in sori but with undivided pinnules; *Thyrsopteris*, peculiar in the extreme dimorphism of sterile and fertile parts of the frond; also *Dennstaedtia* and sometimes other genera.

Mettenius (1856) was the first person to unite all these genera in one family, Cyatheaceae, excluding *Dennstaedtia* from it; in this he was followed by Diels (1899) and Christensen (1905). Bower, however (1926: 326, 333), stressed the significance of marginal as against superficial sori (though he recognized the "phyletic slide" from marginal to superficial sori in *Microlepia* and other genera) and placed Cyatheaceae (s. str.) and Dicksoniaceae on two opposing branches of his phyletic scheme. He included *Dennstaedtia* and related genera (*Microlepia*, etc.) in Dicksoniaceae. Christensen later (1938) followed Bower in making this separation, and Copeland (1947) differed only in including Bower's Dicksoniaceae in a larger family Pteridaceae.

In arriving at his arrangement showing a wide separation ("long overdue") between Cyatheaceae and Dicksoniaceae Bower was much influenced by his association of Cyatheaceae with Gleicheniaceae, certainly a more ancient family (p. 304). In Gleicheniaceae the sori are superficial on the lower surface and have no indusia. Bower's conclusion was that the exindusiate condition is primitive in Cyatheaceae; this involves the further conclusion that the indusia present in other members of Bower's Cyatheaceae are a new development (pp. 304, 307). In this, Bower disagreed with Goebel (1915-18, pp. 1148, 1149), who had regarded the indusia in *Cyathea* as homologous with the indusia in *Dicksonia*. Bower's hypothesis of the indusium in Cyatheaceae (s. str.) as a new development has the further consequence that the indusia in *Dryopteris* are not homologous with those of the Davallioid ferns; yet species of *Davallodes* (a Davallioid genus unknown to Bower) have been included in *Dryopteris* because of the form of their sori.

This postulated lack of homology between extremely similar indusia seems to us highly improbable. A re-examination of *Cyathea*, *Dicksonia*, and their related genera is clearly necessary for a re-assessment of the argument. Such a re-examination has been made, as regards external morphology in the main by Holttum, as regards anatomy and microscopic details of soral development by Sen. Our conclusion is that *Dicksonia*, *Cyathea* and allied genera form a natural group, which should constitute a family Cyatheaceae as in the works of Mettenius and Diels; and that the indusia of *Cyathea* are strictly homologous with those of *Dicksonia*. In view of Bower's association of *Dennstaedtia* and other genera with *Dicksonia*, an examination of *Dennstaedtia* has also been made, which indicates that it should be excluded from the family, though this conclusion does not deny some relationship between *Dennstaedtia* and Cyatheaceae.

Anatomy of Stem and Petioles

A full comparative statement on the anatomy of all the genera will be published elsewhere (by Sen). Here the more important features are briefly noted.

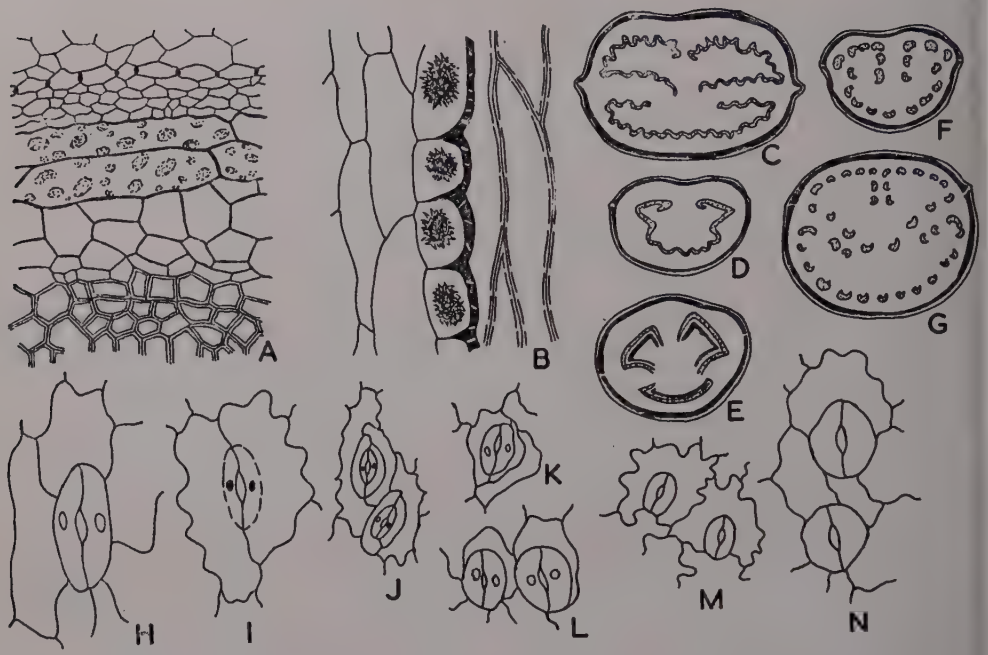
On the massive trunks of *Cyathea* and *Dicksonia* the leaves are arranged in a series of close spirals. Within is a hollow cylinder of vascular tissue in which are gaps corresponding to the leaf-bases, so that a cross-section shows a number of meristeles, each of which has outward-curving ends. On the outer and inner sides of each meristele are plates of very hard sclerotic tissue. Small vascular strands arise from the margins of the gaps to supply the leaves, and in *Cyathea* there are also small medullary bundles which anastomose with each other and also with the meristeles. Distinctive " cubical cells " (Fig. 1B) form a more or less continuous layer surrounding each mass of sclerenchyma; these cells are short (not elongate as the cells of the sclerenchyma), have their walls adjacent to the sclerenchyma and to each other much thickened, and in the cavity of each cell is a crystalline mass which is insoluble in sulphuric acid and may consist of silica. In the outer part of the phloem are " tangential cells " (Fig. 1A) which are like sieve-tubes but are elongated tangentially; they sometimes contain mucilage. Mucilage-sacs are always present in the ground tissue of the stem and petioles.

The numerous vascular bundles near the base of a petiole, as seen in transverse section (Fig. 1G, F), are arranged in three groups, one group forming a broad arc convex to the abaxial side, with inflexed ends, the other two groups, on the adaxial side, like a figure 7 and its mirror image. Above the base of the petiole the separate bundles join together, those of the two adaxial groups joining at their abaxial ends, thus forming two arcs across the petiole, both convex on the abaxial side. Higher in the rachis there may be further simplification of the pattern.

The structure of the stem and petiole is also similar in *Lophosoria*, *Thyrsopteris* and *Culcita*, but in these the stem is less massive, with fewer leaf-gaps in a single cross-section, and in some cases it is prostrate; the petioles are smaller, and the vascular tissue in them forms a single continuous band as seen in cross-section (Fig. 1C, D). In *Thyrsopteris* there are medullary bundles, some of which close the leaf-gaps in a peculiar way; in *Thyrsopteris* also the cubical cells are distributed irregularly and usually singly throughout the sclerenchyma, both in cortex and the pith. The cubical cells are also solitary in *Lophosoria*; in *Culcita* they are in a continuous layer, as in *Dicksonia*. So far as we are aware, cubical cells are found in no other fern genera except *Dennstaedtia*, and tangential cells only otherwise in *Osmunda*.

In *Culcita macrocarpa* the petiolar strand, as seen in cross-section, has its ends divergent, not turned inwards towards each other; but in *C. dubia* (placed by Maxon in a distinct subgenus *Calochlaena*) the ends are inflexed in the normal manner. We have not seen sections of petioles of the other species of *Culcita*, but it seems probable that this anatomical distinction corresponds with the subgeneric division made by Maxon on external characters.

The genera *Cibotium* and *Metaxya*, very different from each other in many ways,



FIGS. 1A-N — Anatomy of stem and petioles. A, *Cyathea costalisora*, t.s. of the inner part of the stem showing tangential cells outside the phloem layer. × 100. B, *Cyathea pulcherrima*, l.s. of a part of the stem showing a layer of cubical cells lying between parenchyma and sclerenchyma. × 264. C, *Thyrsopteris elegans*, t.s. of a petiole. × 2. D, *Metaxya rostrata*, t.s. of petiole. × 3. E, *Lophosoria pruinata*, t.s. of a petiole. × 1. F, *Cibotium barometz*, t.s. of a petiole. × 5. G, *Cyathea dealbata*, t.s. of a petiole. × 1·5. H, *Thyrsopteris elegans*, stoma in surface view. × 200. I, *Lophosoria pruinata*, a sunken stoma. × 170. J, *Metaxya rostrata*, stomata with three subsidiary cells. × 90. K, *Cibotium barometz*, stoma with three subsidiary cells. × 150. L, *Dicksonia squarrosa*, stomata with a single subsidiary cell. × 140. M, *Cyathea contaminans*, stomata with a single subsidiary cell. × 125. N, *Culcita macrocarpa*, stomata with or without a subsidiary cell. × 160.

agree in lacking bands of sclerenchyma surrounding the meristeles, and in absence of cubical cells, though they possess tangential cells in the phloem. They also agree in a more complex formation of stomata. In other genera there is only one subsidiary cell, surrounding (or nearly surrounding) each stoma (Fig. 1L, M), and sometimes no cell has this position (Fig. 1H, N); in *Cibotium* and *Metaxya* there are normally three subsidiary cells (Fig. 1J, K), all formed by division of one cell before the stoma itself is differentiated.

Upper Surface of Axes of Fronds, and Form of Leaflets

In *Cyathea, Lophosoria, Dicksonia* and *Cibotium* the upper surface of each pinna-rachis, and of the costa of each pinnule, is raised, the decurrent bases of the edges of the lamina forming a more or less developed wing along the sides of the axes (Fig. 2A); if in large pinnae the rachis is somewhat grooved (as is the main rachis), this groove is not interrupted by the insertion of the pinnules.

In *Culcita* and *Thyrsopteris* the upper surface of each pinna-rachis, and of smaller rachises (the fronds being at least tripinnate) is grooved, the edges of the groove being raised, and this groove is open to admit the grooves of lesser axes at their insertion (Fig. 2B). The midrib of each leaflet is a groove with raised edges, these edges being decurrent on the edges of the groove of the rachis bearing the leaflet, and

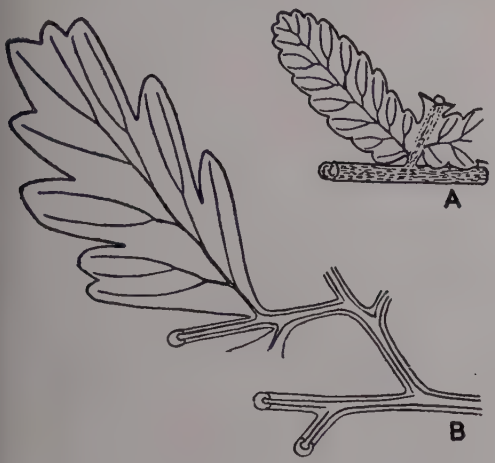


FIG. 2— A, *Cyathea* part of pinna-rachis (upper surface raised and hairy) and base of pinnule. B, *Culcita*, a quarternary leaflet with grooved branches of rachis. Both, × 2·5.

the edges of the lamina are separately decurrent as lateral wings.

In *Cyathea, Lophosoria, Dicksonia* and *Cibotium* the branching of the frond (with few exceptions) is bipinnate, and each pinnule is almost symmetrical in shape (Fig 2A), as in the fossil form-genus *Pecopteris*. In *Culcita* (Fig. 2B) and *Thyrsopteris* the fronds are more amply divided and each leaflet is conspicuously asymmetric, being broad and deeply lobed to the base on its acroscopic side, narrowly cuneate and entire near the base on its basiscopic side, as in the fossil form-genus *Sphenopteris*.

In *Metaxya* the fronds are simply pinnate with almost entire pinnae in the adult condition of the plant. The rachis is grooved on the upper surface, the groove open to admit the grooves of the costae of the pinnae. In young plants the pinnae are rather deeply lobed, and they are distinctly asymmetric at their bases. It appears possible, therefore, that *Metaxya* is derived from ancestors having the leaf-form of *Culcita*.

Hairs and Scales

The protective dermal appendages in genera except *Cyathea* are hairs, which vary much in length, thickness and rigidity in different species and on different parts of a single leaf. In some cases these hairs are branched. Similar hairs occur also in *Cyathea* (Fig. 3A-F), but they are rarely as long as in the other genera; in *Cyathea* the principal protective appendages are scales.

As indicated by Holttum (1957) two types of scales occur in *Cyathea*; they are called flabelloid and setiferous scales. A division of the genus *Cyathea* (including *Hemitelia* and *Alsophila*) according to scale-characters, whether flabelloid or setiferous, appears to be a natural main division of the genus (at least as regards the species of Asia, Malaysia, Australasia and the Pacific, all of which have been examined), whereas a division according to indusium-characters is not. These scales are shown in their fullest development on the bases of petioles (there may be small scales mixed with the larger ones in this position), those on more distal parts of the fronds being gradually smaller; in the branched parts of the frond, scales are present especially on the abaxial surfaces, hairs always on the adaxial surfaces and less commonly on the abaxial ones.

The largest scales of flabelloid type consist mainly of elongate thick-walled cells (usually dark in colour but sometimes quite pale) with more or less wide marginal bands of short thin-walled cells spreading outwards fan-wise, the outer cells forming irregular lobes which end in single elongate cells which in some species have thick walls like those of the central band (Fig. 3U).

The largest scales of setiferous type consist entirely of rather uniform elongate parallel cells, usually not so much thickened nor so dark as the scales of flabelloid type, with more or less abundant obliquely projecting elongate dark thick-walled cells (setae). In some cases the setae may be concolorous with the rest of the scale, and those towards the base may be curved outwards.

Both types of scale can be shown to originate from a single superficial cell, in exactly the same way as hairs, but the subsequent divisions of a scale are more complex than those of a hair, and the mode of development in the two types of scales is different. In *Cyathea contaminans* (Wall.) Copel. and allied species, which

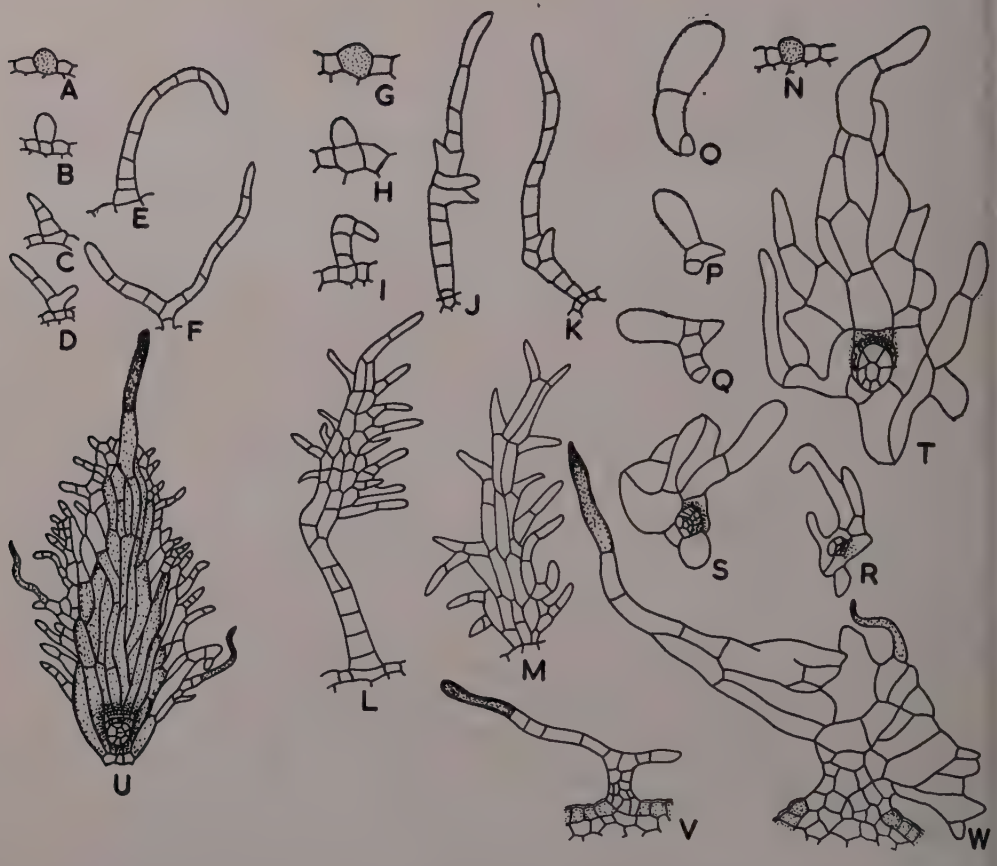


FIG. 3 — Development of hairs and scales. A-F, *Cyathea contaminans*, stages in the development of hairs. G-M, *C. contaminans*, stages in the development of scales showing uniseriate condition during ontogeny. N-U, *C. orientalis*, stages in the development of scales showing uniseriate peltate condition during ontogeny. V-W, *C. orientalis*, vertical sections of flabelloid scales showing peltate base. A, G and N, × 100; B-F, H-M, O-W, × 50.

have setiferous scales, it is easy to see that the smaller scales on the rachis are gradually narrowed towards the base, which is quite terete and in smaller scales consists of a single row of cells, exactly like the base of a thick hair. Figure 3G-M shows stages in the development of small scales of this kind. In the case of larger scales, with thicker bases, the basal cells divide longitudinally to form a more or less conical structure which remains (and may even continue to develop) after the scale has fallen; these conical projections sometimes form strong spines several millimeters long.

Flabelloid scales develop in a more complex way, forming peltate structures at right angles to the base, which soon thickens to a multicellular peg-like structure (Fig. 3N-W). The scales develop much more acroscopically than basiscopically, so that they are very asymmetric about the peltate base, but quite broad basal growths can be seen in some species, notably in the tropical American *Cyathea arborea* (L.) Sm.

Sori

DEVELOPMENT — In view of the considerable difference in the position of a mature sorus as between *Cyathea* on the one hand and *Dicksonia*, *Culcita* and *Cibotium* on the other, it is important to

know what differences exist in the developmental stages. Sections of very young leaflets of *Cyathea* are found to show a condition very little if at all different from those of *Dicksonia* and *Cibotium*. Such sections (Figs. 4A-D, 6B) show the rudiment of the receptacle of the sorus almost symmetrically placed between two outgrowths, one of which is somewhat more massive than the indusium-rudiment and grows much more rapidly in *Cyathea*; but even in *Dicksonia* and *Cibotium* (Fig. 6A) the edge of the leaf (which forms the outer indusium) is from the beginning more massive than the (inner) indusium. The difference in soral position is thus due to later developments, not to the initial divisions of cells at the leaf-margin. It is thus clear that the indusium in *Cyathea* originates in the same way as the indusium in *Dicksonia*, not in the manner of a scale as postulated by Bower. Even in *Gleichenia*, where Bower indicates an entirely superficial origin of the sorus (1899, Fig. 10), some of Bower's own preparations indicate that at an early stage the receptacle-primordium is almost, if not quite, marginal.

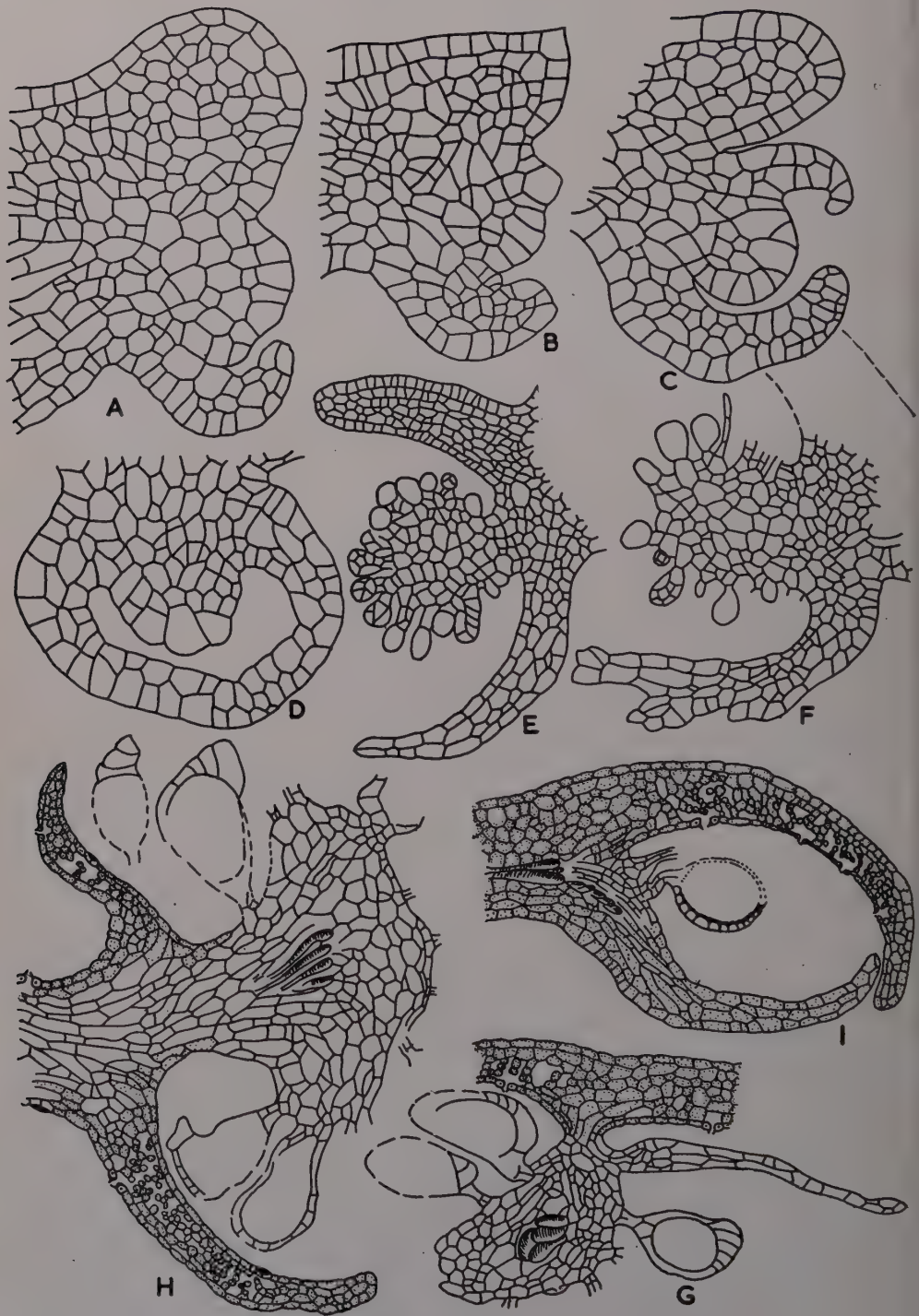
Receptacle — Apart from position, the mature receptacle, which bears the sporangia, is different in another way in *Cyathea* as compared with *Dicksonia*, *Culcita* and *Cibotium*. In *Cyathea* the receptacle is erect and knob-like or columnar, always circular in transverse section (Fig. 4E, F). The sporangia are borne evenly all round it, and are short-stalked. In *Dicksonia*, *Culcita* (Fig. 4I) and *Cibotium* the inner indusium is fused at its base with the receptacle, so that only the acroscopic side of the receptacle is free; this is only slightly raised. There is thus less room for the sporangia in these genera, and the rather long stalks of the sporangia are probably to be correlated with this condition.

In *Thyrsopteris*, though the sorus is strictly marginal, it has a receptacle shaped as in *Cyathea*, and the sporangia are likewise short-stalked (Fig. 4H). In *Lophosoria* the receptacle is slightly raised, round in transverse section; the sporangia have short stalks. In *Metaxya* the receptacle is slightly raised and often more or less elongate along a vein; there may be more than one sorus on a vein. Again, Metaxya shows a highly specialized condition.

Indusia — Only in *Thyrsopteris* are the adaxial and abaxial parts of the indusium identical (Fig. 4H), both containing green cells with intercellular spaces and both with stomata; the two together ultimately form an almost symmetrical shallow cup. In *Dicksonia* and *Culcita* the adaxial (outer) indusium is like a continuation of the leaf-lamina, but somewhat thinner, and has green cells with air-spaces, and stomata on its abaxial surface; the abaxial (inner) indusium is thinner, has little green tissue and few intercellular spaces, stomata being present in *Dicksonia antarctica* but not in *Culcita macrocarpa* (Fig. 4I). In *Cibotium* the initial stages of development are as in *Dicksonia*, but the mature indusia, both outer and inner, are pale (almost devoid of chlorophyll) and have no internal air-spaces; stomata occur occasionally on the outer indusium.

The indusia in *Cyathea* are of much more varied form, and the traditional division into cup-shaped indusia (*Cyathea* proper), asymmetric indusia (*Hemitelia*) and no indusia (*Alsophila*) does not give an adequate statement of this variety. In many species the indusium has never been carefully examined, as shown by the fact that some species still known as *Alsophila* have in fact small indusia, hidden by the mature sori. We have not studied in detail the species of tropical America; the following statement refers only to the species of the Old World, which have all been examined by Holttum (about 400 have been described, but there has been considerable duplication).

As explained below, we believe that the *Hemitelia* type of indusium is primitive in Cyathea, and we will, therefore, begin with this; it is to be noted that in the Old World hemitelioid indusia have only been found on species which have flabelloid scales. In *C. heterochlamydea* Copel., a Philippine species, the indusium is attached only on the side of the receptacle remote from the margin but is quite large (Fig. 5A), and covers the developing sorus almost to maturity. In size and position it comes very near an inner indusium of *Dicksonia*, but differs in not being at all adnate to the receptacle except at its very base.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The *Hemitelia* type of indusium is found in many species of Malaysia, in some being smaller than in *C. heterochlamydea*, in some larger. The smaller indusia form a continuous series down to a very small size [as in *C. latebrosa* (Wall.) Copel. Fig. 5G], and so lead to a completely exindusiate condition (Fig. 5H). The larger hemitelioid indusia have only a small opening on the side towards the margin (Fig. 5C), and have in many cases been mistaken for cup-shaped indusia; examples are *C. oinops* Hassk. and *C. spinulosa* Wall.

Another type of development from the hemitelioid condition is for the attachment of the indusium to spread round the base of the receptacle, thus leading to a saucer-shaped or cup-shaped condition. In the species *C. javanica* Bl. (Fig. 5D) most indusia are almost symmetrically saucer-shaped when old, but on some fronds one can find asymmetric indusia, almost to the hemitelioid condition. Other species, in Sumatra and Borneo, appear to show the transition even more clearly.

There are a considerable range of species which have saucer- to cup-shaped indusia. Those with rather deep cups sometimes break a little when they are old, but when the sorus has just reached maturity, they show quite even edges (Fig. 5E). A deeply cup-shaped indusium such as that of *C. orientalis* (Kze) Moore covers the young sorus except for a very small apical opening; as the sporangia enlarge, the opening gradually expands until the perfect cup-shape is produced. In other species the almost ripe sori look very like those of *C. orientalis*, the indusium with a small apical opening (or sometimes the edges are folded over each other so that no opening appears) but at maturity a cup is not formed (Fig. 5F); dehiscence occurs by irregular breaking of the indusium, the parts persisting if they are firm, or mostly disappearing if they are very thin (as in *C. crenulata* Bl.). Old sori of the latter type have an irregular disc surrounding the base of the receptacle. In other species there is a small disc (quite hidden by the sorus) with even rim, as in *C. batjanensis* (Chr.) Domin; this looks like a reduction from the condition of *C. javanica*, but might also be derived from the condition of *C. latebrosa*, by spreading of the small indusium round the base of the receptacle.

In the Old World species of *Cyathea* which have setiferous scales, we have another series of indusium-forms. Here the hemitelioid condition has not been observed, and one cannot be sure what was the primitive condition of the sorus. Some species have thin indusia which completely cover the young sori (e.g. *C. integra* J. Sm., *C. moluccana* R. Br.), breaking at maturity, never cup-shaped (Fig. 5I). Other species have no indusium whatever [*C. squamulata* (Bl.) Copel., Fig. 5J]. In a few cases intermediate conditions have been found, and these look like hybrids. The best-known is *C. alternans* Wall. (Malay Peninsula and Borneo), which comprises a series of forms intermediate in leaf-form and in indusium between the fully indusiate simply pinnate *C. moluccana* (placed in *Schizocaena* by Copeland) and the bipinnate exindusiate *C. squamulata* (placed in *Gymnosphaera* by Copeland). Every stage of reduction of the indusium has been found, associated with many different intermediate types of frond-form.

In *C. fugax* v. A. v. R. and other species allied to the exindusiate *C. contaminans*, especially in New Guinea and the islands of the Pacific Ocean, there are narrow pale scales radiating round the base of the receptacle of a mature sorus (Fig. 5K); probably they help to protect the young sorus. In one group of species, distinct because of the fully tripinnate form of the fronds, such scales are more

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FIG. 4 — Sorus. A-D, *Cyathea dealbata*, stages in the development of indusia in vertical sections. × 220. E-G, *C. capensis*, stages in the development of indusia. E, F, × 100; G, 90. H, *Thyrsopteris elegans*, v.s. through a mature sorus. × 110. I, *Culcita macrocarpa*, v.s. through a mature sorus. × 92.

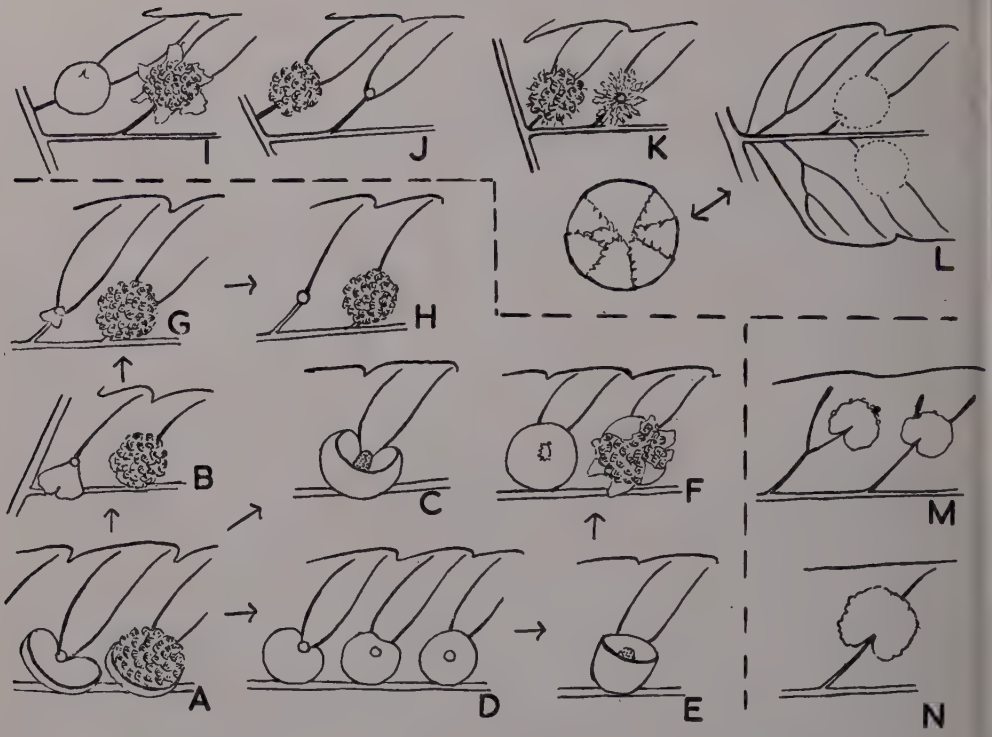


FIG. 5 — A-L, sori of *Cyathea* spp., somewhat diagrammatic, all except L, × 10; arrows indicate probable evolutionary change, starting from A. A, *C. heterochlamydea*, a complete sorus and one with sporangia removed. B, *C. capensis*. C, *C. oinops*. D, *C. javanica*, all sporangia removed, showing indusia only. E, *C. orientalis*. F, *C. crenulata*. G, *C. latebrosa*; H, *C. glabra*. I, *C. integra*, a ripe sorus before and after dehiscence. J, *C. squamulata*, exindusiate. K, *C. fugax*, sorus on right shows scales which surround base of receptacle. L, *C. tripinnata*, with enlarged sorus covered by overlapping scales, true indusium lacking. M, N, *Thelypteris*; M, *T. singalensis*; N, *T. viscosa*, indusia believed to be homologous with those of *Cyathea*.

fully developed, and form a complete cover for the sorus, to maturity (Fig. 5L). Species of this group are *C. celebica* Bl. and *C. tripinnata* Copel.; the indusial scales overlap so closely that they appear to form a continuous indusium very like that of *C. integra*, and have been so described. These indusial scales are quite similar to other small scales on the costules of the lobes of the leaflets, whereas true indusia, where present in *Cyathea*, are quite different from scales, in substance, in superficial cells and in mode of origin.

Copeland (1947) adopted Blume's genus *Gymnosphaera*, in which he associated species with flabelloid scales [*C. glabra* (Bl.) Copel., etc.] with exindusiate species having setiferous scales (*C. squamulata*, etc.). In our view, the latter are closely related to the indusiate setiferous species above mentioned, with which they share a peculiar form of venation and with which there is evidence that they hybridize; no intermediate between *C. glabra* and *C. squamulata* have been found. A statement on the subdivision of the genus *Cyathea*, as regards Malaysian species, will be presented by Holttum in Flora Malesiana, Series II (Pteridophyta), part 2.

Sporangia

We have not made a detailed examination of sporangia in these genera, but in

all the sporangia are of similar general form, and in all cases the number of spores is usually 64; that is, the number is the same as in a majority of the more highly evolved leptosporangiate ferns. The short stalks of the sporangia of *Cyathea* and *Thyrsopteris* are to be correlated with the prominent receptacles of these genera, which allow a larger number of sporangia to develop than would be possible with short stalks on the slightly raised receptacles of *Dicksonia* and *Culcita*. Similarly, the sporangia in Hymenophyllaceae and Loxsomaceae, on elongate receptacles, are short-stalked.

The annulus in all cases is oblique, not interrupted by the stalk, or slightly interrupted in *Culcita*, *Metaxya* and *Cystodium*. The stomium is most highly organized in *Cyathea* and *Metaxya*; in the others it is of various form. *Lophosoria* appears to have the least specialized type of sporangium (Bower, 1926, Fig. 551, p. 287) but even here there is a definite stomium, though apparently not of constant form. Bower states that there are four cells in a transverse section of the stalk of a sporangium of *Cyathea* (he so figures *Alsophila excelsa*, Fig. 563) but six cells can be seen in sections of the stalks of sporangia of *C. dealbata* in Bower's own preparations. The number of rows of cells in all genera is four or more. We do not see that there is any sharp distinction in this character between *Cyathea* and *Dicksonia*, as implied by Bower on p. 308. But we are of opinion that a new comparative survey of sporangia in the genera here treated is desirable (Bower saw few species of *Cyathea*). Clearly we have here a somewhat primitive group of genera, each of which is specialized in various ways along its own lines, and it is not to be expected that the sporangia of all will have evolved to the same extent. The most primitive-looking sporangia in the group are those of *Lophosoria*, but the sori of this genus are considerably evolved, having lost their indusia and developed a fully superficial position.

Spores

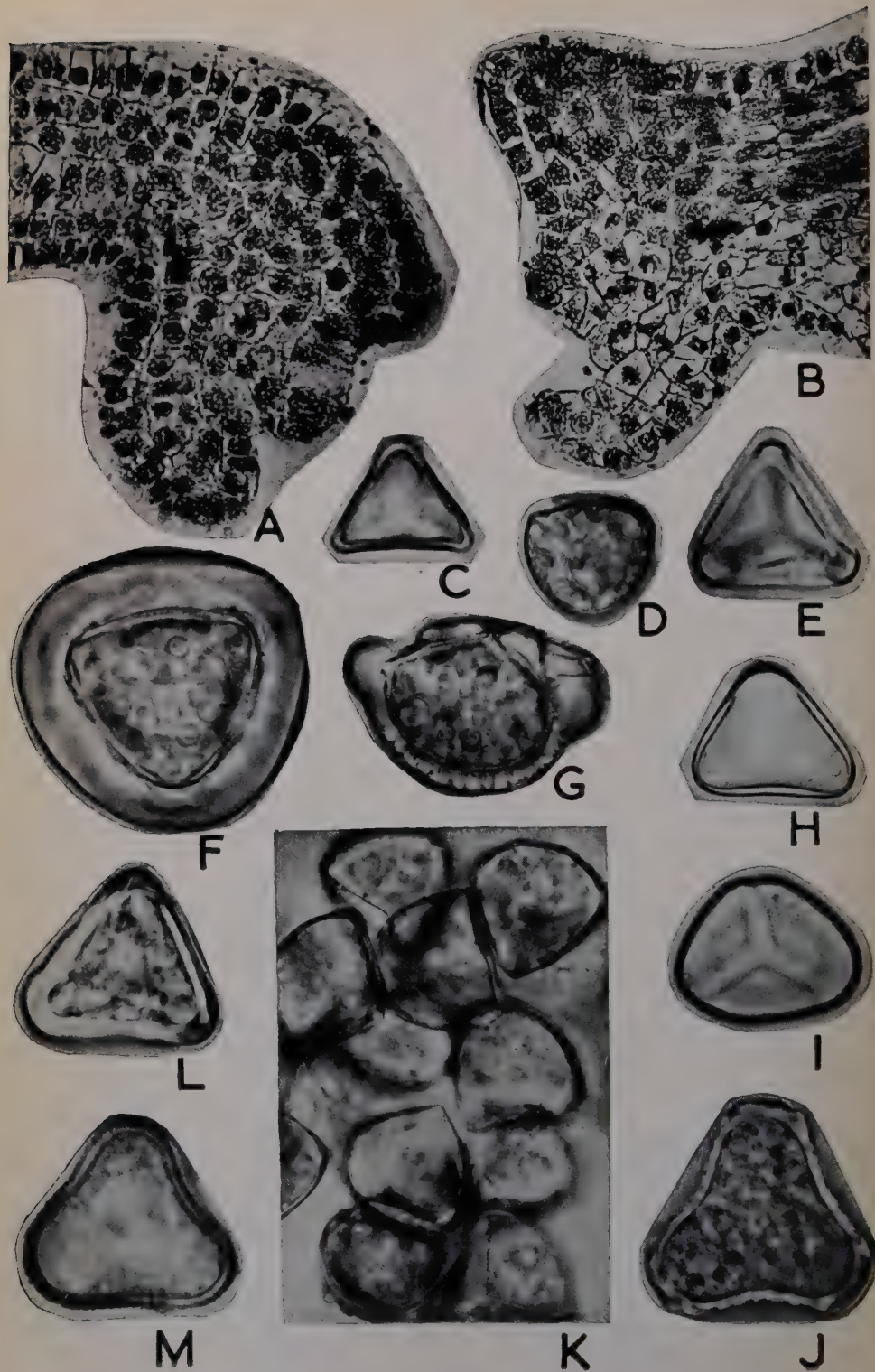
The spores in all genera are trilete, the exine smooth or papillose (Fig. 6). In *Dicksonia* the walls are thickened at the angles (the spore viewed from the polar side), in *Cyathea* of even thickness throughout. In the genus *Cnemidaria* the walls of each face as so viewed are thickened, and there is a small circular hollow in the middle of each face. For this reason, and because of simply pinnate fronds with a peculiar type of anastomosis of veins, we consider that *Cnemidaria* should be regarded as a distinct genus (the free-veined species included by Copeland in *Cnemidaria* do not have this type of spore and in our opinion should be excluded). In *Lophosoria* the spores are peculiar in having a continuous thickened band all round the circumference, as seen in polar view (Fig. 6F, G).

The Genus *Dennstaedtia*

This genus was included in *Dicksonia* by Hooker, in *Synopsis Filicum*, and also by other authors, for which reason we include a note upon it here, to show why we exclude it from *Cyatheaceae*. Hooker's reason for including it in *Dicksonia* was the position of the sorus at the end of a vein and protected by a cup-like indusium which is sometimes slightly two-lipped at the mouth; he argued that there is no sharp distinction here from *Dicksonia*, as in the latter genus the two indusia (outer and inner) are somewhat joined at the base.

Dennstaedtia has a relatively slender dorsiventral rhizome which is often wide-creeping; this contrasts with the radially organized and shorter more massive prostrate stems which occur in some species of *Culcita* and *Cibotium*. Anatomically, cells resembling the cubical cells of Cyatheaceae occur in association with the sclerenchyma of *Dennstaedtia*, in small groups, about as in *Thyrsopteris*, but silica bodies have not been observed in them. No tangential cells occur in the phloem of *Dennstaedtia*, nor are mucilage-sacs present.

The receptacle in *Dennstaedtia* differs from that of *Dicksonia*, *Culcita* and *Cibotium* in being columnar, free from the inner indusium; in this it resembles *Thyrsopteris*. The sporangia are more highly evolved than in any genus of Cyatheaceae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(as here construed); they always have an interrupted annulus with a well-defined stomium.

Dennstaedtia appears to be closely related to other genera with rather slender, long-creeping rhizomes, namely *Microlepia*, *Hypolepis*, *Paesia*, *Pteridium* and perhaps others, and in our opinion further morphological and anatomical studies of these genera are needed to establish clearly their limits and inter-relations. It is notable that in this group of genera, as in Cyatheaceae, both types of rachis-structure appear (both occur in *Dennstaedtia* itself; *see* Tryon 1960) and that there appear to be intermediate conditions in *Microlepia* and *Hypolepis*. The distinction between the *Pecopteris* and *Sphenopteris* types of frond-form also appears to be less sharp than in Cyatheaceae.

Thus we are of opinion that though there is evidence of some relationship between *Dennstaedtia* and Cyatheaceae, it is better to treat *Dennstaedtia* as a member of another group of genera. Whether this group should have the rank of a family, or whether it is better regarded as a subfamily of a larger family, are questions which we cannot attempt to answer at present.

Cytology

Chromosome-numbers observed in the genera *Dicksonia*, *Cibotium* and *Culcita* have been recorded by Manton (1958, p. 84) and in *Cyathea* by Manton & Sledge (1953) and by Manton (1954); no observations are yet available on *Thyrsopteris*, *Lophosoria* and *Metaxya*. The numbers are: *Dicksonia*, 65 (three species); *Culcita macrocarpa*, 66-68; *Cibotium*, 68 (two species); *Cyathea*, 69 (several species). These numbers indicate that an association of these genera in one family is not unreasonable, though the variety in the numbers indicates, as do characters of gross morphology, that the genera have become considerably differentiated during the course of evolution from a presumed common ancestor.

Culcita dubia (R. Br.) Maxon has recently also been examined by Prof. Manton, who permits me to report that the haploid chromosome number is about 55. Maxon placed this species, with the few others from Australaisia-Malaysia, in a distinct subgenus *Calochlaena*. It would be possible to rank *Calochlaena* as a separate genus, but the morphological distinctions are not great, and we incline to maintain the subgeneric status.

The chromosome-numbers of *Dennstaedtia* (34, 64-65, 94), *Microlepia* (43, 86, 129) and *Hypolepis* (52, 104) are more diverse; but the strong morphological resemblances between these genera leave us in no doubt that they are nearly allied to each other. *Paesia* (n = 26) is closely related to *Hypolepis*, and is interesting because it retains the inner indusium, which has disappeared in *Hypolepis*. *Pteridium*, like *Hypolepis*, has the number 52; but *Pteridium* is much more specialized in many ways, and *Hypolepis* is far nearer to *Dennstaedtia* in general morphology than to *Pteridium*, in spite of the very different chromosome numbers.

Discussion

Considering first the principal genera *Cyathea* and *Dicksonia*, we believe that Bower's wide separation between them was unwarranted, in view of the close similarity of early stages in the development of sori in the two genera. A difference not noted by Bower (or other previous authors we have seen) is that in *Dicksonia* there is a fusion between the inner indusium and the receptacle, along with a lateral extension of the base of the

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FIG. 6 — Photomicrographs. A, *Cibotium schiedei*, vertical section of a young sorus. × 55. B, *Cyathea dealbata*, vertical section of a young sorus. × 55. C-M, Spores. × 500. C, *Cyathea contaminans*. D, *Metaxya rostrata*. E, *Thyrsopteris elegans*. F, G, *Lophosoria pruinata*. H, I, *Culcita macrocarpa*. J, *Dicksonia squarrosa*. K, *Cyathea costalisora*. L, *Cibotium barometz*. M, *Cyathea pulcherrima*.

inner indusium. A free columnar receptacle, as in *Thyrsopteris* (also seen in *Loxsoma* and in Hymenophyllaceae), would appear to have been the primitive condition, and this is retained by *Cyathea*. If this argument is correct, then *Cyathea* has evolved separately from a time before *Dicksonia* developed the fusion of receptacle and indusium.

Having included *Dicksonia* and *Cyathea* in one family, we can proceed to an assessment of the other genera which have been associated with them.

The association of a special upper-surface structure of the axes with markedly *Sphenopteris*-form of the lamina in both *Thyrsopteris* and *Culcita* causes us to associate these two genera, though they differ in the condition of the receptacle, in which *Culcita* agrees with *Dicksonia*. If we are right in associating these genera, then the same form of receptacle and indusium evolved separately in *Culcita* and *Dicksonia*. If *Culcita* were placed next to *Dicksonia*, we should need to assume independent origin of a very peculiar rachis-sturcture and frond-form in *Thyrsopteris* and *Culcita*.

Lophosoria appears to be the sole representative of a line which developed superficial sori, much as in *Cyathea*, with loss of inner indusia, but without evolving scales as a protection of the young parts of the plant. Its paraphyses are much like those of *Dicksonia*.

Cibotium and *Metaxya* differ so much from each other, and also from the other genera, that we put each in a separate sub family, though they have in common a very peculiar method of formation of stomata. *Metaxya* is much specialized in its simple pinnae and in the occurrence of more than one sorus on a vein (sori also often elongate along the veins). The simplification of leaf-form makes the rachis-structure less obvious, but in this, and possibly in *Sphenopteris*-type of leaf-form, we incline to think that *Metaxya* shows characters in common with *Thyrsopteris* and *Culcita*.

Subdivision of Cyatheaceae

Subfamily CYATHEOIDEAE

Fronds normally bipinnate; pinnules almost symmetrical (*Pecopteris*-type); upper surfaces of pinna-rachis and costae of pinnules raised; sori terminal on veins or on lower surface of veins, indusiate or not; dermal appendages hairs or scales or both; cubical cells present in association with sclerenchyma; stomata with single subsidiary cells.

Tribe *Cyatheae*

Scales and hairs present as dermal appendages; sori superficial, indusiate or not; cubical cells in continuous layer on surface of sclerenchyma.

Fronds mostly bipinnate; veins free (with very few exceptions); spores with thin walls of uniform thickness; indusia various ... ... *Cyathea*

Fronds simply pinnate; veins regularly anastomosing; spores with wall much thickened, a round hollow in the wall in the middle of each face; indusia hemitelioid ... ... *Cnemidaria*

Tribe *Lophosorieae*

Hairs only as dermal appendages; sori superficial, no indusia; cubical cells singly in association with sclerenchyma. ... ... *Lophosoria*

Tribe *Dicksonieae*

Hairs only as dermal appendages; sori marginal, protected by a slightly modified marginal lobe of the lamina (outer indusium) and a thinner inner indusium; receptacle of sorus fused to inner indusium.

Frond bipinnate with deeply lobed pinnules, or tripinnate; stem normally a thick erect trunk; cubical cells as *Cyathea* ... ... *Dicksonia*

Frond bipinnate with simple pinnules; stem prostrate; condition of cubical cells not yet investigated ... ... *Cystodium*

Subfamily THYRSOPTERIDOIDEAE

Fronds 3-4-pinnate, leaflets of *Sphenopteris*-type; upper surfaces of axes and of leaflet-midribs grooved, grooves of major axes open to admit those of minor ones; sori at ends of veins; cubical cells present; stomata with single subsidiary cell.

Tribe *Thyrsopterideae*

Fertile and sterile parts of frond strongly dimorphous; receptacle of sorus columnar with sporangia all round it; indusium ultimately a shallow cup; stem massive, erect; cubical cells scattered ... ... *Thyrsopteris*

Tribe *Culciteae*

Fertile and sterile parts of frond not greatly dissimilar; receptacle of sorus fused to inner indusium (as in *Dicksonia*); inner indusium thinner than outer, the two slightly joined together at their bases; stem in most species prostrate; cubical cells in a continuous layer as in *Cyathea* ... ... *Culcita*

Subfamily CIBOTIOIDEAE

Fronds normally bipinnate; pinnules almost symmetrical; upper surfaces of rachises and costae raised; sori terminal on veins, shaped much as in *Dicksonia* but with outer and inner indusia both unlike the lamina of the frond, lacking chlorophyll and lacking intercellular spaces; cubical cells lacking; stomata with three subsidiary cells ... ... *Cibotium*

Subfamily METAXYOIDEAE

Fronds simply pinnate, pinnae lobed on young plants only; upper surface of rachis and midribs of pinnae grooved, groove of rachis open to admit grooves of pinnae; sori superficial on lower surface of veins, usually more than one to a vein, no indusia; cubical cells lacking; stomata with three subsidiary cells ... *Metaxya*

Summary

A comparative statement is given concerning external morphology and anatomy of the genera *Cyathea, Dicksonia, Cystodium, Culcita, Cibotium, Thyrsopteris, Lophosoria* and *Metaxya*. It is concluded that, contrary to most recent taxonomic arrangements, these genera are sufficiently closely allied to form a natural family, a tentative conspectus of which is given. Further information is needed concerning *Cystodium*. A statement is given concerning the forms of scales and of indusia to be found in the Old World species of the genus *Cyathea*, which is treated as including *Alsophila, Hemitelia, Gymnosphaera* and *Schizocaena*. The genus *Cnemidaria* (tropical America) is regarded as distinct from *Cyathea*.

We are grateful to Dr C. D. Adams for supplying material of *Lophosoria* from Jamaica, and to Dr K. U. Kramer for material of *Metaxya* from Suriname. Material of several species of each of the genera *Cyathea, Dicksonia, Cibotium* and *Culcita* was mainly obtained from living plants in the fern-houses at the Royal Botanic Gardens, Kew, and at the Botanic Garden, Glasgow; we wish to record our thanks for permission to use this material. Some data have also been obtained from herbarium specimens, chiefly at Kew, and from microscopic preparations in the collections of the Department of Botany, University of Glasgow.

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REVIEWS

STILES, WALTER 1961. "Trace Elements in Plants." University Press, Cambridge. Pp. 249. Price 40s.

For the last 16 years, Professor Stiles' book has been the main source of reference and consultation for plant physiologists in the field of trace elements. It is, therefore, a pleasure to see its third and latest edition.

Some changes have been made in the organization of the book. Previously the title had been "Trace Elements in Plants and Animals". Animals have now been dropped from consideration excepting a chapter on "Trace elements in plants in relation to some diseases of grazing animals". Even in the older edition, not more than about a fifth of the space was devoted to animals and Professor Stiles has rightly left them to be taken care of by zoologists. The book has been enlarged with about 60 new pages, and in the list of references, there appear about 150 new entries published since 1948. There are two new chapters, "The effects on plants of trace-element excess" and "Factors influencing the absorption of trace elements and their effects on plants". Under yet another chapter on trace element deficiency diseases, there appears a new section on the symptoms of chlorine deficiency, obviously in recognition of mounting evidence of the essentiality of this element. A welcome feature is also the inclusion of, a dozen or so, new photographs of various deficiency symptoms.

There are a few misprints. For example, throughout the book the name Agarwala is spelled Argawala. Another author appears as Boxma in the text and Boxima in the index. Some elements on which one might expect some discussion in the text, such as fluorine or iodine, are not even listed in the index. It is also curious that among the new references incorporated in the reference list, there are 22 published in the year 1956, 8 published in the year 1957, 6 published in 1958, and only 2 published in the year 1959 (the manuscript was sent to press in 1960). However, these minor criticisms do not reflect on the otherwise high standard of the book. Both in style and contents, the book stands high along with the author's other works, "Introduction to Plant Physiology", and "Respiration" (jointly authored with Leach), which have been used by students all over the world.

Satish C. Maheshwari

DAVIES, DAVID D. 1961. "Intermediary Metabolism in Plants." University Press, Cambridge. Pp. 108. Price 20s.

Mr Davies' little volume, the latest in the Cambridge series of monographs in experimental biology, is just about the right size of a book that one would like to read in these days of an ever expanding volume of literature. The last comprehensive text that appeared in this field is James Bonner's "Plant Biochemistry", published in 1950. In the decade that has passed, many notable advances have been made. Our knowledge of the carbon reduction cycle in photosynthesis, photosynthetic phosphorylation, hexosemonophosphate shunt pathway for oxidation of sugar, role of acetyl-CoA, ribosomal protein synthesis, and more recently the contributions in mechanism of nucleic acid synthesis, reflects only a small portion of the progress made in recent years.

The author is, therefore, to be congratulated for attempting to bring together the scattered literature under the fold of just two covers. There are 7 chapters in the book. The first on "Metabolic Patterns and Cellular Organization" is followed by "Organization and Structure", "Bioenergetics", "Catabolism", "Anabolism", "Links between Metabolic Pathways", and finally "Conclusion". These chapters embrace almost all aspects of plant biochemistry with the exception of photosynthesis and nucleic acid metabolism, the latter topic having been omitted on account of lack of adequate

work on it. A good deal has, however, been done even in this field since the author sent his manuscript to press. If a new edition is brought out, it is hoped that the author will add a new section to cover developments in this area too.

The choice and treatment of topics are rather personal. The fact that the book is organized on the basis of a lecture series is very obvious even without reading the author's preface. As the author says, " presentation of factual knowledge requires encyclopaedic treatment " on account of the very rapid progress in this discipline. What he set out to do is to give a bird's-eye view of the major developments and to outline the concepts and theories of intermediary metabolism. With the limitation set by space, he has no doubt done an admirable job. His style is distinctive, and the treatment lucid. The numerous sketches, the list of over 200 references, and the index are very welcome features of the book. There is no doubt that every student of plant biochemistry and plant physiology will want to have a copy on his shelves.

SATISH C. MAHESHWARI

SALTON, MILTON J. 1960. " Microbial Cell Walls." John Wiley and Sons, Inc. New York and London. Pp. 94. Price $ 3.50.

THIS handy volume is the outcome of lectures given by the author at Rutgers in the Institute of Microbiology, State University of New Jersey, U.S.A., under the CIBA programme of annual lectures in microbial chemistry.

Speaking generally, cell wall chemistry, in plants and in other organisms, has proved to be a difficult subject for investigation because of the great heterogeneity of cell walls and technical difficulties arising by their insolubility in common solvents. The problems one faces in bacteria are even tougher. The microbial cell is versatile as novel and unique cell-wall components, rare linkages, and unusual isomers abound in them. Consequently, enzymic and other methods of hydrolysis, which have enabled us to understand the structure of many other types of polymer molecules, are relatively impotent as analytical tools in their case.

Nevertheless, the unusual chemistry itself provides special stimulus for investigations in this field. The development of the techniques of electrophoresis, chromatography, centrifugation and fractionation, in the past decade, has enabled the unfolding of at least some of the secrets. Although at this date the information is much too fragmentary and not amenable to a generalized account, the book is very welcome because it brings together for the non-specialist the highlights of recent research reported in scattered papers.

There are just 3 chapters in the book. Chapter 1, entitled " Isolation and general properties of microbial cell walls ", outlines the basic features of the cell wall and the knowledge gained by electron microscopy. Numerous electron photographs, well-chosen and well-reproduced, complement the description. Chapter 2 is on the " Chemistry of cell walls ". Both in this and the following chapter the yeasts and the fungi get separate, though brief, treatment. The account of bacteria is further subdivided, the gram negative bacteria following the gram positive. Mention is made of the elemental composition of the cell wall and the chemical nature and percentages of the monosaccharides, amino sugars, teichoic acids, and amino acids obtained on breakdown. The account also brings to light the unusual occurrence of D-isomers of amino acids and the work done on the identification of N-terminal and C-terminal amino acid residues by the DNP technique. The last and the most interesting chapter is that on " Enzymic degradation and biosynthesis of microbial cell walls ". Various enzymes such as chitinases, mannases and N-acetyl hexosaminidase, found in the wall are mentioned. Finally, there is a discussion of the steps in the biosynthesis of the cell wall with the help of radioactive precursors, and the identification of uridine and cytidine-linked intermediates, evidence for which has also appeared for higher plants.

The author says in conclusion, " There is little doubt that next ten years will see an enormous widening of our understanding of both the chemical structure and biosynthesis of these fascinating struc-

tures". The book will certainly help to attract the attention of many potential investigators to this field.

The publishers deserve to be congratulated for the fine get-up and printing.

SATISH C. MAHESHWARI

MAHESHWARI, P. & VASIL, VIMLA 1961. "Gnetum." Botanical Monograph No. 1, Council of Scientific & Industrial Research, New Delhi. Pp. 142 + xii, plates 2, figures 86. Price Rs. 20 (40s.)

THIS is a fine work, both well and clearly written and illustrated. What was known about *Gnetum* has been reviewed in the light of new and intensive investigation. There can be no further excuse for omitting this Amphioxus of botany from university syllabuses. That this work has come from India is a good sign. Not only does it show the high standard which the senior author has there established, but that most progress in morphology will come from tropical botany. *Gnetum*, so vast and tardy in its liane-lengths, must as others have found be studied by those with continual access to the plants. We must be most grateful to the Council of Scientific & Industrial Research, New Delhi, for starting such a promising series of monographs.

Two points occur to me in this review. Firstly, in a case like this of a small genus, subgeneric classification and a key to the species would be welcome. Secondly, small size, so admirably maintained in this volume, makes a better seller and a better reader. Therefore much purely descriptive matter could be compressed succinctly into descriptive phraseology so that it is at once available for consultation. Morphology, I think, has still to learn this economic discipline of taxonomy; and the text is more available for thought.

E. J. H. CORNER

BRIGGS, G. E., HOPE, A. B. & ROBERTSON, R. N. 1961. "Electrolytes and Plant Cells." Blackwell Scientific Publications, Oxford. Pp. 270+x. Price 40s.

THE new series of "Botanical Monographs" edited by Professor W. O. James are a welcome addition to the growing literature on plant sciences. "Electrolytes and Plant Cells", the first volume in this series, is a joint endeavour of three active and able researchers in the field — Briggs, Hope and Robertson. The authors aim not to provide exhaustive information but to deal with general principles emanating from recent research investigations. The treatment of the subject is acceptable both to the botanist and the physical chemist.

The book is divided into four parts. Part I entitled "The properties of electrolytes and plant cells" concerns the physical aspects like diffusion and properties of electrolytes. The discussion of Donnan's theory and its bearing on different plant systems is a good inclusion here. There are two chapters on the structure of plant cells. One gives the gross structure of cells, especially the internodal cells of *Chara* which have been extensively used as experimental material. The drawings are somewhat amateurish and the legends brief. The fine structure of cells has been beautifully illustrated with electron micrographs, mostly from Professor F. V. Mercer of Sydney University and his collaborators.

Not much information is available on ion absorption by cellular organelles like the nucleus, plastids and mitochondria. However, the authors have adequately discussed the relation of the fine structure and the properties of the organelles with the process of salt absorption. The evaluation of the model lipoprotein structure of a hypothetical membrane proposed by Danielli and Davson from the point of view of the requirements of a plant membrane is critical and illuminating

Part II has been devoted entirely to the concept of free space, its volume, measurement and electrical potential differences. The problems arising in the estimation of the free space are fully described and will be of considerable interest from the practical standpoint.

"Metabolic interactions between cells and electrolytes" is the subject-matter of Part III. This is perhaps the most interesting section to a biologist since it relates to metabolism and ion absorption. A concise account of the flux measurements

in single cells and tissues which are so important in understanding the interaction between cells and ions forms the first chapter of this part. Also included here is the curious effect of light on the process of accumulation.

Since the classical experiments of Hoagland, Steward, Lundegårdh and Davis in the 1920's and 1930's interest has centred around the relation between respiration and ion absorption. There is a concise account of this subject in Chapter 15. No clear picture of the exact relation between active transport and respiration has, however, emerged. A critical summary of the various theories of the mechanism of active transport forms the last chapter of this part. It is concluded tentatively that it is the anion which is transported actively while the cation moves along the electrochemical potential gradient.

Part IV has a somewhat indifferent heading "Electrolytes in tissues and plants". Some very interesting aspects are discussed here, for example, the uptake of ions as influenced by the stage of growth and ion absorption in entire organs and salt glands. Quantitative studies of ion absorption by the entire organism or even its organs is often cluttered due to ignorance of the sites and rates of growth of the component tissues and cells. Contrary to earlier concepts, it now appears that it is the mature, vacuolated cells (not the young and enlarging cells) that show ion accumulation at a faster rate. The high uptake of cations in the meristematic cells is not true accumulation but the result of an increase in the indiffusible anions.

This book will be of special assistance to plant physiologists as an introduction to the principles and problems of ion absorption in plants. One of its merits is the excellent bibliography containing 207 references, out of which nearly 60 per cent are works published since 1950. However, some references quoted in the text have not been included in the bibliography. Among these may be mentioned: Burström (1933, 1935); Vervelede & Tendeloo (1953); Weiber (1952).

One obvious drawback in this handy and neatly printed volume containing numerous graphs, tables and figures is the highly abbreviated index making it difficult to locate many authors' names and plant names. A more thoughtful choice could have been made of some headings and sub-headings, likely to be searched by the reader. Some of the key ommissions in the index are: bromide, caesium, carrot, hydrogen ion concentration or pH, Michaelis constant, *Nitella*, symplast, *Vallisneria* and *Valonia*.

On the whole, the book is a stimulating and useful compilation for the student of cell physiology.

H. Y. Mohan Ram

Botanical Monograph No. 1

GNETUM

P. MAHESHWARI & VIMLA VASIL
Department of Botany
University of Delhi, India

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